Stem-end Chip Defect in Potatoes (*Solanum tuberosum* L.)

By

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At the University of Wisconsin-Madison

Abstract

Global consumption of potato (*Solanum tuberosum* L.) continues to shift from fresh potatoes to value-added processed food products such as potato chips. One serious tuber quality defect of chipping potatoes is stem-end chip defect, which results in chips with dark-colored vasculature and adjacent tissues on the tuber stem end after frying at a high temperature. The influences of moderate water stress, alone or in combination with moderate heat stress, as well as maturity of tubers at harvest on the incidence and severity of stem-end chip defect were investigated. Treatments were imposed in controlled-environment greenhouses. Only temperature stress, daytime temperature at 30°C, in one of two years significantly changed the occurrence of stem-end chip defects. Biochemical analyses showed that more severe defects were associated with increased amounts of tuber stem-end glucose and increased stem-end acid invertase activity. Moderate environmental stresses and immaturity of tubers at harvest, however, were not sufficient to consistently cause stem-end chip defects.

Verticillium wilt, caused by a soil-borne fungal pathogen *Verticillium dahliae,* is a

persistent potato disease that causes early plant senescence and yield reductions. A two-year field trial has been conducted to investigate the effects of *V. dahliae* on stem-end chip defect development and the underlying biochemical mechanism. Conditions that favored vascular infection with *V. dahliae* were more likely to cause a high incidence of severe stem-end chip defects than those that were less favorable for infection. Infection of *V. dahliae* was associated with an up-regulation of acid invertase activity and an accumulation of reducing sugars on the tuber stem end. High concentrations of reducing sugars are the direct cause of dark color on chips since reducing sugars produce dark-colored products in the Maillard reaction during frying.

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Processed potato products are consumed worldwide

Potatoes rank as the world's third most important food crop after wheat and rice. Potatoes are consumed fresh, processed into potato food products and food ingredients, fed to livestock, processed into starch for industry, and re-used as seed tubers for growing the next season's potato crop. Food and Agriculture Organization of the United Nations (FAO) estimates that just over two-thirds of the 320 million tons of potatoes produced in 2005 were consumed by people as food in one form or another. Fresh potatoes are baked, boiled or fried and used in a staggering range of recipes: mashed potatoes, potato pancakes, potato salad, etc (International Year of the Potato 2008). But global consumption of potato as food is shifting from fresh potatoes to value-added, processed food products. One of the main items in that category is potato chips. Potato chips are the long-standing king of salty snack foods in many developed countries (International Year of the Potato 2008). The 2009 Snack Food Statistics showed that the annual sales for the snack food industry in the US was $29 billion, and potato chips accounted for $7 billion, followed by tortilla chips and corn snacks, $5.5 billion and $0.9 billion respectively (https://www.aibonline.org/resources/statistics/2009snack). Made from thin slices of potato and deep-fried or baked, they come in many different flavors - from simple salted to "gourmet" varieties tasting of roast beef and Thai chili, for

example. Some chips are produced using dough made from dehydrated potato flakes (International Year of the Potato 2008).

History of the potato chip industry

The birth of potato chips

Potato chips did not gain widespread market appeal in the United States until the mid 1900s. They were invented in the 1850s when a chef humorously responded to a customer's complaint that the eating establishment's fried potatoes were cut too thick (Talburt 1975). It wasn't until the introduction of automatic potato-peeling machines in the 1920s, the emergence of small chip companies in the 1930s, along with subsequent innovations in frying techniques in the 1950s that potato chip production began its rise to mass-consumption popularity (Lucier and Dettmann 2008). During World War II, potato chip consumption grew rapidly and the industry emerged as a major user of potatoes (Love *et al.* 1998). Twelve percent of the potatoes produced in North America in 2002 were processed into chips (National Potato Council).

Chipping potato marketing: price, supply chain, transportation, and warehouse storage

Potato chip consumption in the United States has steadily increased over the past five decades from 11.4 pounds per person in 1960 to an estimated 15.9 pounds per person in 2007 (Table 1.1, U.S. Potato Statistics (91011)). Monthly retail price for potato chips increased from $1.98 per pound in January 1980 to $3.33 per pound in December 1999,

Table 1.1: US per capita utilization of potatoes, 1960-2007. Potato chip consumption in the United States has steadily increased over the past five decades from 11.4 pounds per person in 1960 to an estimated 15.9 pounds per person in 2007.

	Pounds per capita, farm weight						
Year	**Total**	**Fresh**	**Total**	**Processing**			
				Freezing	**Chips & shoestrings**	**Dehydrating**	**Canning**
1960	106.3	81	25.3	7.6	11.4	4.9	1.4
1961	108.3	80.7	27.6	9.1	11.9	5.1	1.5
1962	104.2	76.1	28.1	9.3	12.5	4.8	1.5
1963	110.5	78.6	31.9	11.9	13.4	5.1	1.5
1964	108.6	74.8	33.8	12.3	14.5	5.4	1.6
1965	109.9	66.7	43.2	18	15.5	8	1.7
1966	118.8	71.7	47.1	18.9	16.3	10.2	1.7
1967	108.5	60.4	48.1	20.2	16.4	9.8	1.7
1968	116.2	65.3	50.9	22.1	16.6	10.4	1.8
1969	117.8	62.6	55.2	24.9	17.1	11.3	1.9
1970	121.7	61.8	59.9	28.5	17.4	12	2
1971	117.8	56.1	61.7	30.1	17.2	12.3	2.1
1972	119.4	57.9	61.5	30.3	16.7	12.4	2.1
1973	118.2	52.4	65.8	34.2	16.3	13.1	2.2
1974	117.2	49.4	67.8	35.3	15.7	14.5	2.3
1975	121.9	52.6	69.3	37.1	15.5	14.7	2
1976	125.2	49.4	75.8	41.8	15.8	16.3	1.9
1977	122.1	50.1	72	42.2	16.2	11.4	2.2
1978	119.5	46	73.5	42.6	16.5	12.1	2.3
1979	117.8	49.3	68.5	38.5	16.7	11.2	2.1
1980	114.7	51.1	63.6	35.4	16.5	9.8	1.9
1981	116.5	45.8	70.7	41.5	16.6	10.8	1.8
1982	115	47.1	67.9	38.6	17	10.4	1.9
1983	118.7	49.8	68.9	39.2	17.8	10	1.9
1984	122.1	48.3	73.8	43.7	18	10.3	1.8
1985	122.4	46.3	76.1	45.4	17.6	11.2	1.9
1986	125.9	48.8	77.1	46.3	18.1	10.9	1.8
1987	126	47.9	78.1	47.9	17.6	10.8	1.8
1988	122.3	49.6	72.7	43.3	17.1	10.4	1.9
1989	127	50	77	46.8	17.4	10.8	2
1990	123.9	46.7	77.2	46.4	16.4	12.6	1.8

1991		134.1	50.2	83.9	51.1	17.3	13.8	1.7
1992		129.9	48.3	81.6	49.9	17.1	12.8	1.8
1993		136.7	50.1	86.6	53.5	17.7	13.7	1.7
1994		136.7	49.6	87.1	55.7	16.5	13.2	1.7
1995		136.9	49.2	87.7	56.2	16.4	13.2	1.9
1996		145	49.9	95.1	60.2	16.4	16.7	1.8
1997		137.8	47.3	90.5	57.8	15.5	15.5	1.7
1998		137.7	46.9	90.8	58.1	14.7	16.5	1.5
1999		136.2	47.7	88.5	58.5	15.9	12.4	1.7
2000		137.9	47.1	90.8	57.5	15.9	15.7	1.7
2001		138.6	46.5	92.1	58.1	17.6	14.8	1.6
2002		131.9	44.2	87.7	55.1	16.5	14.7	1.4
2003		138.2	46.8	91.4	57.2	17.3	15.5	1.4
2004		134.6	45.8	88.8	57.3	16.5	13.8	1.2
2005		125.5	42.4	83.1	53.6	16	12.6	0.9
2006	p	123.7	42	81.7	52.7	16.2	11.9	0.9
2007	f	126	43.6	82.4	52.9	15.9	12.7	0.9

p = Preliminary. f = Forecast.

Source: Economic Research Service, USDA.

and entered into a relatively stable stage between $3.30 per pound and $3.60 per pound between 2000 and 2007 (Table 1.2, U.S. Potato Statistics (91011)). Retail prices for potato chips are considerably higher than other potato products (such as fresh or frozen French fries). Since 2000, retail prices for potato chips have averaged $3.41 per pound, while fresh and French fried potatoes have averaged $0.45 and $1.05 per pound (U.S. Potato Statistics (91011)). Frying oil is an expensive input in chip production, contributing to high retail chip prices. Because chips are thinly sliced and contain more surface area, they absorb more oil than other processed products such as French fries. Depending on the type of frying oil used, oil content in chips can range between 30 and 40 percent (Warner *et al.* 1994). It takes a large amount of potatoes to produce potato chips. A potato is roughly 80 percent water (when harvested) and it requires about 4

pounds of raw potatoes to manufacture 1 pound of potato chips (Lucier and Dettmann, 2008).

The traditional potato chip industry has greatly consolidated operations over the past 20 years, with fewer plants processing greater portions of the chip supply. In 1960 there were 400 plants processing 907 million kg (20 million cwt) of chipping potatoes (Table 1.3, U.S. Potato Statistics (91011)). In 2006 there were 92 chipping plants processing 2.7 billion kg (67 million cwt) of chipping potatoes – a 68 percent growth in production with 77 percent fewer processing plants. Most plants are strategically located around large population centers in the United States because long-distance transportation of fried chips is uneconomical. The largest share of potato chip processing plants (37 percent) is located in the eastern States, processing 42 percent of the potatoes used for chipping. The remainder of the potato chip industry is located in the Midwest (32 plants) and Western States (26 plants) (Lucier and Dettmann, 2008).

Potato chips are made from tubers delivered daily to manufacturing plants (Lucier and Dettmann, 2008). The sources vary from season to season. In April and May, potatoes come from Florida; June, July and August bring potatoes from North Carolina and Virginia; in the autumn months, potato growing states in the Northern part of the US supply the majority of potatoes; during the winter, French fry and chip manufacturers depend on stored supplies of potatoes (Lucier and Dettmann, 2008).

Potatoes for chip processing are usually bought from the grower or storage warehouse under the stipulation that if the potatoes do not fry well, they will be returned.

Table 1.2: Potato Chips: US monthly retail price, 1980-2007. Monthly retail price for potato chips increased from $1.98 per pound in January 1980 to $3.33 per pound in December 1999, and entered into a relatively stable stage between $3.30 per pound and $3.60 per pound between 2000 and 2007.

Year	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Annual
							Dollars per pound						
1980 1/	1.98	1.99	2.00	2.01	2.01	2.02	2.01	2.05	2.04	2.07	2.14	2.18	2.04
1981	2.20	2.20	2.23	2.28	2.27	2.30	2.26	2.29	2.30	2.32	2.38	2.34	2.28
1982	2.38	2.41	2.39	2.38	2.40	2.39	2.39	2.42	2.40	2.43	2.39	2.40	2.40
1983	2.44	2.49	2.51	2.52	2.54	2.47	2.48	2.52	2.54	2.50	2.52	2.53	2.50
1984	2.49	2.56	2.52	2.52	2.56	2.56	2.58	2.60	2.61	2.63	2.59	2.58	2.57
1985	2.60	2.61	2.61	2.63	2.62	2.61	2.62	2.60	2.56	2.60	2.60	2.63	2.61
1986	2.61	2.61	2.63	2.62	2.62	2.71	2.64	2.72	2.70	2.75	2.74	2.75	2.68
1987	2.75	2.79	2.81	2.80	2.78	2.80	2.79	2.72	2.70	2.67	2.73	2.67	2.75
1988	2.63	2.60	2.60	2.58	2.60	2.57	2.62	2.62	2.64	2.62	2.66	2.70	2.62
1989	2.71	2.88	2.91	2.95	2.94	2.88	2.84	2.86	2.82	2.82	2.86	2.85	2.86
1990	2.87	2.95	2.96	2.98	2.94	2.95	2.95	2.95	2.98	3.03	2.97	2.97	2.96
1991	2.99	2.98	2.95	2.96	2.94	3.06	2.93	2.94	3.02	2.93	2.95	2.96	2.97
1992	2.94	2.91	2.94	2.86	2.87	2.91	2.94	2.97	2.86	2.94	2.84	2.84	2.90
1993	2.83	2.94	2.84	2.84	2.90	2.83	2.89	2.97	2.90	2.91	2.84	2.92	2.88
1994	2.87	3.02	2.97	3.01	3.00	2.97	2.90	2.99	2.97	2.92	3.02	3.01	2.97
1995	3.08	3.01	2.98	3.05	2.95	2.99	2.97	3.04	2.97	3.02	2.98	3.03	3.00
1996	3.00	2.96	3.04	3.08	2.99	2.98	3.11	3.09	3.07	3.16	3.10	3.12	3.06
1997	3.13	3.10	3.15	3.18	3.06	3.12	3.12	3.13	3.18	3.15	3.13	3.17	3.13
1998	3.15	3.14	3.11	3.18	3.16	3.15	3.13	3.21	3.12	3.22	3.21	3.18	3.16
1999	3.22	3.22	3.25	3.26	3.21	3.24	3.26	3.28	3.24	3.29	3.30	3.33	3.26
2000	3.39	3.45	3.35	3.41	3.35	3.30	3.31	3.30	3.42	3.34	3.28	3.44	3.36
2001	3.39	3.36	3.38	3.32	3.40	3.56	3.34	3.40	3.40	3.59	3.51	3.48	3.43
2002	3.44	3.28	3.42	3.29	3.16	3.43	3.20	3.31	3.50	3.36	3.42	3.47	3.36
2003	3.48	3.46	3.55	3.58	3.42	3.58	3.37	3.54	3.51	3.43	3.49	3.58	3.50
2004	3.43	3.58	3.44	3.38	3.59	3.45	3.60	3.46	3.24	3.40	3.31	3.35	3.43
2005	3.26	3.32	3.22	3.46	3.38	3.52	3.36	3.36	3.30	3.37	3.41	3.46	3.37
2006	3.43	3.37	3.52	3.46	3.60	3.37	3.47	3.54	3.53	3.95	3.40	3.41	3.50
2007	3.36	3.42	3.49	3.48	3.51	3.47	3.51	3.61	3.56				3.49

-- = Not available.

1/ Data not available prior to 1980.

Source: Bureau of Labor Statistics, U.S. Department of Labor (CPI Average Price Data; U.S. City Average Potato Chips).

Table 1.3: Utilization of US potatoes, 1959-2006. There was a 68 percent growth in potato chip production with 77 percent fewer processing plants from 1959 to 2006.

Crop year	Number of plants 1/	Quantity used for		
		Potato chips	French fries	Potato chips and French fries 3/
	- Number -		-- 1,000 cwt --	
1959 2/	413	19755	461	20216
1960	400	20593	592	21185
1961	372	22609	604	23213
1962	366	24083	717	24800
1963	358	26477	728	27205
1964	361	28093	671	28764
1965	342	30968	690	31658
1966	327	32015	762	32777
1967	314	32107	922	33029
1968	307	33238	961	34199
1969	295	35061	1060	36121
1970	278	34283	774	35057
1971	272	35112	679	35791
1972	261	33788	759	34547
1973	242	33921	778	34699
1974	228	32200	780	32980
1975	213	33306	801	34107
1976	205	34374	592	34966
1977	201	36874	485	37359
1978	228	37333	506	37839
1979	181	37548	728	38276
1980	175	37131	763	37894
1981	169	38587	759	39346
1982	168	40119	657	40776
1983	163	42764	547	43311
1984	155	41752	587	42339
1985	156	41697	520	42217
1986	165	45375	439	45814
1987	166	40210	383	40593
1988	164	43958	581	44539
1989	156	42719	352	43071

1990	156	44186	133	44489
1991	148	45389	461	45850
1992	147	47764	691	48455
1993	141	48354	633	48987
1994	131	48683	616	49299
1995	126	47834	550	47284
1996	122	47814	491	48305
1997	119	47527	603	48130
1998	113	51032	472	51504
1999	109	52445	496	52941
2000	99	52143	262	52405
2001	99	53885	195	54080
2002	99	51445	195	51640
2003	98	52236	518	52754
2004	93	49874	194	50068
2005	91	50998		50998
2006	92	67034		67034

1/ Number of potato chip plants in the U.S.

2/ Data prior to 1959 is not available

3/ Includes shoestrings to avoid disclosure of individual firms

Source: "Potatoes", National Agricultural Statistics Service, USDA.

Therefore, the necessity of a uniform product presents the problem of maintaining desirable chip quality throughout the storage season (Work *et al.* 1981). Tubers must be kept in the most desirable condition for up to nine months during the processing year to provide a uniform flow of supply to the chipping plant through fall, winter and spring, generally from early October to mid-April or longer (Gould *et al.* 1979).

Good quality potato chips require good frying practice

Effects of frying oil composition on potato chip quality

Potatoes are fried in corn oil, cottonseed oil, or a blend of vegetable oils. Oils with lower linolenic acid, higher oleic acid and higher saturation degree have greater frying stability

as judged by less oxidation, polymerization and hydrolysis (Warner *et al.* 1994). However, a major question to be answered is how these fatty acid composition changes affect the flavor of foods fried in the modified oils (Gillatt 2001). Cottonseed oil, with its high level of linoleic acid (52%), is considered to be the industry standard for producing food with desirable fried-food flavor. This flavor may be partly derived from the formation of 2, 4-decadienal during the thermal oxidation of linoleic acid (Warner *et al.* 1994). On the other hand, foods such as potato chips that are fried in high linoleate-containing oils are not oxidatively stable (Warner *et al.* 1994). Antioxidants play an important role in the storage of fried foods, as they prolong the shelf-life of the product. The most commonly used synthetic antioxidants are butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), *tert*-butylhydroquinone (TBHQ) and propyl gallate (PG) (Houhoula *et al.* 2003). TBHQ is not permitted in EU countries and Japan, but is permitted in the USA and some other countries. Nevertheless these antioxidants are sometimes included in commercial frying fats and shortenings and thus extend shelf life of the fried foods (Rossell 2001).

The early potato chip bag was wax paper with the ends ironed or stapled together (http://www.tinynewyorkkitchen.com/potato-chip-history/). At first, potato chips were packaged in barrels or tins, which left chips at the bottom stale and crumbled. Laura Scudder, an entrepreneur in Monterey Park, California started having her workers take home sheets of wax paper to iron into the form of bags, which were filled with chips at her factory the next day (Pepsico). This pioneering method reduced crumbling and kept the chips fresh and crisp longer. This innovation, along with the invention of cellophane,

allowed potato chips to become a mass market product. Today, chips are packaged in plastic bags, with nitrogen gas blown in prior to sealing to lengthen shelf life, and provide protection against crushing (http://www.tinynewyorkkitchen.com/potato-chip-history/).

Chip color is the most important measure of quality

Chip quality is defined by various parameters, and samples are evaluated throughout the manufacturing process (Work *et al.* 1981). Inspectors check the chips for color, seasoning, salt, moisture, breakage by analysis, and for peel, greening, external and internal blemishes visually (Table 1.4). However, the most common index is color. Consumer preference is predominately for an attractive light brown chip (Work *et al.* 1981). Therefore, to meet the preference of the consumer and to produce a superior product, factors affecting chip quality are important to both the grower and the processor and must be well understood by both to maintain acceptable chip color throughout the chipping season (Work *et al.* 1981).

Table 1.4: Various parameters that define good chip qualities. Inspectors check the chips for color, seasoning, salt, moisture, breakage by analysis, and for peel, greening, external and internal blemishes visually.

By analysis	**Visually**
Color	Peel
Moisture	Greening
Salt	External blemishes
Seasoning	Internal blemishes
Breakage	Seasoning coverage
FFA	Blisters
PV	Foldovers
	Oil soaked crisps
	Soft centers/clusters

Development of varieties for good chip color

The production of high quality potato chips with uniform, attractive light color requires stringent quality control measures. Selection of the appropriate cultivars is one method used to meet established specifications (Gould 1980). During the early years of chip manufacturing, cultivars bred specifically for potato chips were not available, and processors used the best existing cultivars. As the industry became better established, varieties were developed specifically for production of potato chips (Cunningham and Stevenson 1963; Thompson 1975). This led to the release of several widely used cultivars during the period extending from 1960 to 1970. Among these were Superior (Rieman 1962), LaChipper (Miller *et al.* 1963), Monona (Stevenson *et al.* 1965), Lenape (Akeley *et al.* 1968), Norchip (Johansen *et al.* 1969), and Shurchip (O'Keefe 1970).

The Maillard reaction causes dark chip color

Changes in the chemical balance, primarily in the content of the reducing sugars glucose and fructose, as well as sucrose content of the potato prior to harvest and during storage, are responsible for subsequent changes in quality of chipping potatoes. The reason that reducing sugar contents can greatly affect chip quality is because of the Maillard Reaction. During frying at high temperature, the carbonyl group on a free reducing sugar can interact with the α-amine group of a free amino acid and, through subsequent reactions, produce advanced glycation products. Examples of compounds produced in the Maillard reaction include Strecker aldehydes and alkylpyrazines, which

are the major flavor compounds of fried potato (Morales *et al.* 2008), and melanoidins that contribute dark brown color (Damondaran *et al.* 2008). The Maillard reaction also produces 2,3-butandione and N-glycosides, which reacts with asparagines or pyrolyzes, respectively, to form acylamide, which is a known neurotoxin and potential carcinogen (Mottram *et al.* 2002; Stadler *et al.* 2002; Friedman 2003). The amount of acrylamide positively correlates to chip color (Silva and Simon 2005; Pedreschi *et al.* 2006). A darker-colored potato chip typically contains more acrylamide than a lighter colored chip (Granda *et al.* 2005).

Factors determining potato chip quality

Varietal differences and agricultural practices affect the sugar level in tubers

Quality of the raw product has been found to vary between and within varieties when grown under different cultural and environmental conditions (Ooko and Kabira 2011). Varietal differences due to variations in genetic makeup and chemical composition (Hoover and Xander 1963; Miller 1972; McCann *et al.* 2010) are known to be responsible for much of the variation in chip quality. Miller *et al.* (1975) reported that the content of the major sugars found in potatoes, glucose, fructose, and sucrose, varied among good and poor chipping varieties. Tuber maturity, sucrose content at harvest, growing season temperatures, soil moisture, fertilization, and postharvest storage are some of the factors that affect the resulting sugar accumulation in newly harvested tubers (Akeley *et al.* 1955; Murphy and Goven 1967; Smith 1975; Sowokinos 1973).

Chemical immaturity should be reduced

Tubers must undergo chemical maturation for optimal chip color. Chemical maturity refers to the sucrose content within tuber tissues (Bussan *et al.* 2009b). The extent of chemical maturity reflects the movement of sucrose within the plant during the growing season (Bussan *et al.* 2009b). Sucrose is a 12-carbon, non-reducing sugar that occupies a critical position in potato tuber development. Translocated to tubers from the leaves, sucrose is the major source of carbon and energy for starch synthesis and potato growth (ap Rees and Morrell 1990). The sucrose concentration is high in immature potatoes because its rate of translocation to the tuber exceeds its rate of metabolism in the tuber. During potato development some of the carbon from sucrose is completely oxidized to CO_2, H_2O and energy (i.e., ATP). Vacuolar acid invertase activity increases in storage, producing undesirable reducing sugars from the available sucrose pool. Therefore, high levels of sucrose in potatoes at harvest can be detrimental to color after a few days in storage (Sowokinos and Preston 2003). When tubers mature, the movement of sucrose to the tubers slows, and thus the concentration of sucrose approaches the lowest levels since it is rapidly converted to starch upon entering into the tubers (Bussan *et al.* 2009b). Chemical maturity is achieved when sucrose concentration reaches a minimum, which is typically 1.0 mg g^{-1} fresh weight (FW) (Bussan *et al.* 2009b). Tubers harvested before they are chemically mature will have relatively high sucrose concentrations when put into storage. Higher sucrose can lead to elevated reducing sugar and thus darker chip color by Maillard reaction when frying, lowering the quality of chips (Bussan *et al.*

2009b).

Monitoring chemical maturity of chipping potatoes has been common practice for several years. Chemical maturity is determined by measuring sucrose content in the tuber tissues. Chipping potato farmers typically start to monitor sucrose concentrations the first week of August and continue sampling every seven to 14 days until the tubers become chemically mature (Bussan *et al.* 2009b). If the sucrose concentration after harvest rises above 1.0 mg g^{-1} FW, the storage manager needs to adjust the storage environment (i.e. temperature and/or ventilation), to remove excess sugar that affects processing quality (Sowokinos and Preston 2003).

The chemical maturity of processing russets potatoes is rarely monitored by commercial growers. Sugar concentrations of the bud and stem ends of russets and other long tubers are typically quite different, so sugar concentrations must be monitored separately in the two ends of tubers. Sucrose concentrations of Russet Burbank (grown in central Wisconsin) were minimized by early to mid-September in both ends of the tuber, with concentrations being higher in the bud end than the stem end. In contrast, glucose concentrations were minimized up until chemical maturity. However, glucose concentrations increased in the stem end of the tuber from the time of chemical maturity until harvest. Bud-end glucose stayed at a lower level during the same time period (Bussan *et al.* 2009b).

Temperature and soil moisture are factors that influence tuber sugar contents

Growing season temperature has a large impact on potato growth and product quality.

The optimum temperature for potato growth and tuber development has been determined to be 24°C daytime temperature and 12°C nighttime temperature (Johnstone 2012). High temperatures, above 29°C, for example, decrease cell division and lower the supply of carbohydrates available to the tuber (Hiller 2002). Previous greenhouse studies indicated that potato grown at intermediate air temperature (16-24°C) had high specific gravity, dry weight, starch content, and tuber yield, in addition to uniform shape and good vine development (Yamaguchi *et al.* 1964). However, those grown at high temperature (27-30°C) had misshapen tubers, chain tubers, high sugar levels, and low starch content. Because heat and drought stress tend to occur together, it has been difficult to separate their roles in affecting tuber qualities for processing product (Thompson *et al.* 2008). The optimum moisture level has been defined as above 65 percent available soil moisture (ASM) (Hiller 2002). The degree of influence of water deficit on tubers is dependent upon the stage of growth of the plant and size of the tuber at the time of stress. Early stress is likely to be much more detrimental to potato quality than late-occurring stress (Iritani and Weller 1973c; Iritani *et al.* 1973b; Iritani 1981; Pavlista and Hergert 2011).

Potato yield and quality can be adversely affected by high temperatures and water stress, or a combination of these, at certain stages of growth. Heat or water stress during early tuber bulking can cause accumulation of reducing sugars in the basal or stem end of the tubers after harvest (Kincaid *et al.* 1993), resulting in dark colors on the stem end portion after processing. When this happens on tubers used for French fries, it is called sugar-end defect.

Sugar-end defect is a persistent quality problem for fry processing potatoes

Sugar-end potatoes can have a dark or "burned" appearance on the tuber stem end because of Maillard reaction after frying (Fig. 1.1). Typical sugar-end potatoes are marked by low starch and high reducing sugars in the older, stem-end tissue while the younger, bud-end tissue has high starch and low reducing sugar content (Eldredge *et al.* 1996; Iritani 1981, 1987; Iritani and Weller 1973, 1980; Kleinkopf *et al.* 1988; Shock *et al.* 1987, 1992, 1993).

Fig. 1.1: Sugar-end defect of potato in cooked French fries (Thompson *et al.* 2008). Sugar-end potatoes can have a dark or "burned" appearance on the tuber stem end after frying.

Sugar-ends in potatoes develop in response to stress. Heat and drought stress, particularly during early tuber bulking, have been implicated in causing dark ends (Hiller and Thornton 1993; Iritani 1981, 1987; Iritani and Weller 1980; Kincaid *et al.* 1993; Kleinkopf 1979; Shock *et al.* 1992, 1993).

Management practices to reduce effects of high soil temperature and soil water stress on tuber sugar accumulation

Increased average soil temperature correlated with more sugar-end tubers in Russet Burbank (Kincaid *et al.* 1993). Stark and McCann (1992) found that irrigation deficits imposed during early to mid tuber bulking caused the highest percentage of sugar-ends in Russet Burbank. Shock *et al.* (1993) examined the timing of a single episode of water stress on potato stem-end reducing sugars and fry color. A single period of water deficit resulting in soil water potentials of -82 to -86 kpa was associated with increases in Russet Burbank tuber stem-end reducing sugars post harvest. In addition, tubers suffering more severe water stress had higher reducing sugar levels after they were harvested and placed into storage. Furthermore, Eldredge *et al.* (1996) demonstrated the increase in stem-end reducing sugar content observed in Russet Burbank tubers in response to soil water potential did not begin within two weeks after the stress ended. Past literature postulated that sugar-ends tubers are a result of soil moisture deficit followed by good growing conditions: starch in the stem end of tubers is broken down and translocated to locations of renewed growth immediately after the stress was removed (Iritani 1973b; Lugt 1960; Nielson and Sparks 1963; Penman 1928; Eldredge *et al.* 1996). However, the delay in the detection of elevated reducing sugar following the relief of transitory water stress suggested that reducing sugars were not produced through starch degradation to provide carbohydrate energy for vine growth or secondary tuber growth during stress. Instead, the delayed reducing sugar increase

could be caused either by sugar translocation from the plant top to the tuber stem end not being incorporated into starch synthesis, or starch in the tuber stem end degraded and sugars accumulated in place, or both (Eldredge *et al.* 1996).

The critical period for tuber quality appears to be from mid-June to mid-July, based on measured soil temperature differences. Increased mid-season soil temperature can increase incidence of tubers that produce dark colored process products. Although soil temperature is primarily controlled by air temperature and radiation, it can be reduced by using more frequent sprinkler irrigation (Kincaid *et al.* 1993). Crop management practices which produce higher percentages of US number one tubers also tend to reduce the incidence of tubers that fry dark (Kincaid *et al.* 1993).

Soil type can affect optimum irrigation management in the field. Potatoes grown on sandy soil required daily irrigation amounts near daily evapotranspiration (ET) to avoid reductions in yield and quality (Hang and Miller 1986). However, high yields and qualities can be obtained on a loam soil with a daily irrigation well below ET during the bulking period if an early irrigation replenished the soil water to field capacity (Martin and Miller 1983; Miller and Martin 1983).

Irrigation frequency and timing of stress can influence potato tuber quality. Miller and Martin (1990) found early varieties performed best with daily irrigation to replace ET, and poorest when irrigation was interrupted during tuber bulking. Ojala *et al.* (1990) found that yield and quality were greatest when the irrigation deficit occurred either early or late in tuber growth, if stress was minimized during the mid-bulking period.

Although appropriate irrigation during the growing season can help reduce moisture

deficit or temperature stress to the plants to prevent tuber quality defects, it should be monitored within an allowed range. Powelson and Christensen (1991) showed that excessive irrigation prior to tuber set will dramatically increase the incidence of *Verticillium* wilt (Vw) in potato induced by the fungus *Verticillium*. Early-season stressed plants had prolonged vegetative vigor in the fall, suggesting that they were less influenced by Vw. Consequently, it is possible that early water stress indirectly influenced the differential proportions of tubers in market classes and the proportion of tubers with dark stem-end fry colors (Shock *et al.* 1992).

Verticillium can significantly affect tuber quality

Verticillium wilt (Vw) is an important vascular disease of potato, which causes early plant senescence and yield reductions of up to 46% in the United States (Davis 2001; Rouse 1985; Rowe 1985; Rowe *et al.* 1987). Vw is caused by the soil-borne fungus *Verticillium dahliae* Kleb in warm production areas and *Verticillium albo-atrum* Reinke & Berthold in cooler regions (Rowe and Powelson 2002). Although *Verticillium* is the primary pathogen associated with Vw, other organisms have been linked to the disease complex. Interactions between *Verticillium dahliae* and the root lesion nematode *Pratylenchus penetrans* appears very common in the US and has been reported to be the primary cause of Vw (Rangahau).

Reduced net photosynthesis and transpiration and increased leaf surface temperature during sunny days are generally the first symptoms of Vw although they are not visible by eye at this stage. The typical visual disease symptoms, acropetal progression of

chlorosis and necrosis of leaves followed by premature defoliation may be indistinguishable from natural senescence, although these symptoms sometimes occur on only one side of the plant or on individual leaves (Isaac and Harrison 1968; Powelson 1993). Vascular browning is often prominent in the bases of affected stems, and necrosis of the vascular ring develops in tubers of some cultivars (Powelson 1993). Advanced symptoms, such as loss of plant vigor during mid to late summer followed by senescence and death of the crop a few weeks prior to normal maturity (Rangahau), usually do not appear until the tuber-bulking stage (Powelson 1993). The specific infection area within the plant vascular systems makes *V. dahliae* a good candidate for investigating its contribution to the development of stem-end chip defect tubers.

V. dahliae is more widespread in the U.S than *V. albo-atrum*. It is common in the north central states and the Pacific Northwest where average summer temperatures frequently exceed 25°C. Consequently, temperature seems to be the factor most influencing the prevalence of *V. dahliae* (Rangahau). A wet season is more conductive to the development of Vw disease than a dry season. In general, Vw is favored by soil moisture and evapotranspiration conditions that result in rapid unrestricted flow of water – and pathogen propagules – through stems and leaves of the plant. After plants senesce and die, *V. dahliae* develops a resting structure called microsclerotia which is composed of compact masses of thick-walled, pigmented cells originating from swollen and separate hyphae by a process of budding (Heale and Issac 1965). Microsclerotia are capable of sustaining the fungus in the soil in the absence of a susceptible host for over 10 years (Schreiber and Green 1963).

In the presence of a susceptible host and under favorable environmental conditions, microsclerotia will geminate into codinia that grow into hyphae (Rangahau). The fungus then invades the plant root system through broken root hairs, of which up to 20% may be found in a healthy plant, and establishes itself in the xylem vessels, spreading upwards into aboveground stems, petioles and leaflets driven by the transpiration pull (Pegg and Brady 2002). However, the mechanism by which conidia enter the underground tuber is not known. In potato plants, conidia are swept up to perforated xylem end walls where, if they do not pass through, they germinate and grow through to sporulate again on the other side (Perry and Evert 1983; Pegg 1985). Why the fungus is confined to the xylem vessels and only rarely found in xylem parenchyma until after the death of the host is unknown. Pegg and Brady (2002) stated that while a good correlation may exist between the location and intensity of xylem colonization with Vw symptom severity in susceptible hosts, the same does not apply to tolerant hosts.

There are two proposed mechanisms for the Vw disease. The first one is that water transport in the xylem of plants is disrupted because stem and petiolar vessels are occluded by *Verticillium* (Johnson and Dung 2010). The other is that the vascular pathogen lives effectively in the xylem vessels and exerts its effects on the host plant by the release of toxic metabolites into the transpiration stream. Toxic molecules, as extracellular metabolites of the fungus, are envisaged to pass through xylem pit membranes into leaf and other parenchyma cells and cause cellular dysfunction and death (Pegg and Brady 2002).

Since fungal structures are not visible on diseased specimens, a laboratory culture is

done to confirm a diagnosis of *V. dahliae*. Small, thin pieces of infected vascular tissue can be placed onto a culture medium such as Sorensen`s NP-10 medium and incubated in darkness for two weeks. When the fungus grows out of the vascular tissues it can be visualized using a microscope. A satisfactory sample for analysis should include several stem or branch segments, with attached leaves, showing a range of symptom severity if possible (Gomez-Alpizar 2001). *Verticillium* assay is also done on soil, primarily for soils in which solanaceous crops such as potatoes, tomatoes, eggplants or peppers are to be grown. The dark microsclerotia that the fungus produces are readily seen in culture plates. It is assumed that the risk of damage for the crop is higher if the *V. dahliae* count is high (Gomez-Alpizar 2001). In potato and the other solanaceous crops the following criteria have been established by Michigan State University per gram of soil:

Low risk 0-5 colonies

Moderate risk 6-12 colonies

High risk >12 colonies

The disease is typically controlled through the use of fumigants, but concerns about the economic and environmental costs of fumigation bring its sustainability into question (Rowe and Powelson 2002). Cultural control practices with a positive impact include crop rotation (Chen *et al.* 1995; Easton *et al.* 1992; O'Sullivan 1978), solarization (Davis and Sorensen 1986; Lazarovits *et al.* 1991), soil amendments (Lazarovits *et al.* 2001; Soltani *et al.* 2002), fertility management (Davis and Sorensen 1986; Davis *et al.* 1990), and irrigation management (Cappaert *et al.* 1992; Cappaert *et al.* 1994; Davis and Sorensen 1986). Sanitation of field equipment to avoid introduction of contaminated

soil and burning or effective removal of vines before harvest are especially important in fields that are planted to potatoes in the first time. Seed tubers destined for new fields should be tested for the presence of *Verticillium* and not planted if infected (Integrated Pest Management for Potatoes in the Western United States). Biological control has potential, but consistent results have not yet been attained (Entry *et al.* 2000; Nagtzaam *et al.* 1998; Sessitsch *et al.* 2004).

Good post harvest storage practices help to produce good quality tubers

According to a recent USAD report, about 17 billion kg (380 million cwt) of potatoes were produced in 2011 in the US, of which 2.4 billion kg (54.7 million cwt), 14% of the total annual yield, were processed for potato chips. To ensure a steady supply of raw product year-round for processing, up to 80% of the potato crop is stored in climate-controlled warehouses. Maximizing the value of stored tubers requires careful crop management going into storage (Bussan *et al.* 2009b). Chemically immature tubers with sucrose level higher than 1.0 mg g^{-1} FW require preconditioning prior to cool-down for long-term storage. It is recommended to hold the temperature of the tubers at the suberization temperature, generally within the range from 12°C to 16°C until sucrose drops to an acceptable level, usually < 0.7 mg g^{-1} FW (Bussan *et al.* 2009b; Forbush). Sometimes excessive sucrose is a result of disease pressure in the field. This type of sugar accumulation is very difficult to remove with preconditioning. Therefore, it is critical to determine the reason for high sucrose content prior to cooling the potato tubers in storage to avoid “locking in” sucrose that cannot be easily removed from the

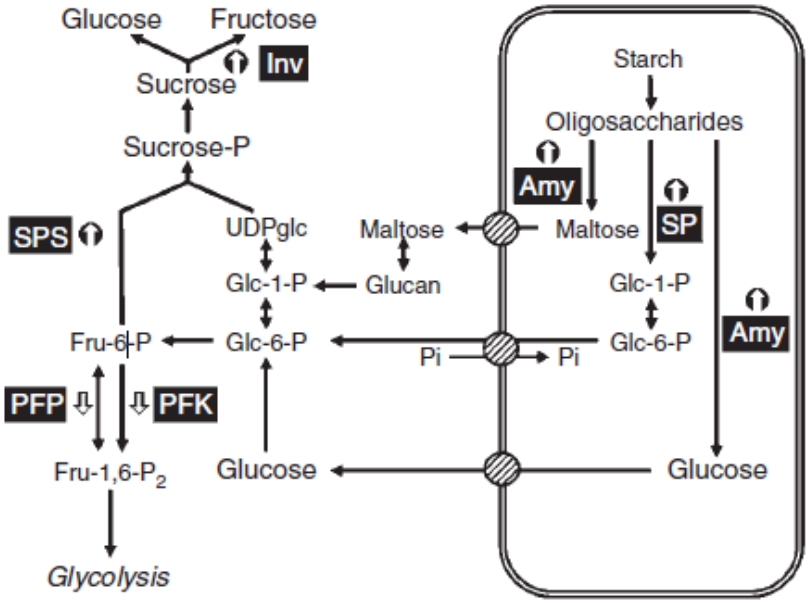
tubers while holding and reconditioning (Forbush). Longer preconditioning periods for chemically immature tubers can lead to increased weight loss and potential for disease development (Bussan *et al.* 2009b).

Carbohydrate metabolism during cold storage of tubers

During storage of tubers, starch is remobilized and converted to sugars while at the same time sugars are recycled to produce starch (Fig. 1.2). Starch may be degraded either hydrolytically by amylase (Amy) or phosphorolytically by starch phosphorylase (SP) and the products exported from amyloplasts either as hexose phosphates (hexose-P) via the glucose phosphate-phosphate translocator or as free sugars via the glucose and/or maltose transporters (Smith *et al.* 2005; Weber 2004).

A range of starch-degrading enzyme activities have been reported in stored potato tubers, including a-amylase, isoamylase, p-amylase, a-glucosidase, and phosphorylase (Schneider *et al.* 1981; Cochrane *et al.* 1991a, 1991b; Cottrell *et al.* 1993). However, it is not clear whether the degradation is primarily amylolytic or phosphorolytic. Morrell and ap Rees (1986) concluded that the degradation of starch in cold-stored potato tubers was phosphorolytic because they detected no amylase activities in the tubers. However, several other authors (Sowokinos *et al.* 1985; Davies and Ross 1987; Cochrane *et al.* 1991b; Cottrell *et al.* 1993) have detected substantial activities. This discrepancy may be due to the use of different assay procedures.

Fig. 1.2: Proposed pathways of carbohydrate metabolism in potato tubers during cold storage (Malone *et al.* 2006). Circled up arrows, indicate reactions catalyzed by an enzyme for which low temperature storage has been reported to result in increased catalytic capacity or enhanced expression of a specific isoform; Down arrows indicate reactions catalyzed by an enzyme reported to be sensitive to direct low temperature inhibition or inactivation. The enzymes indicated are: Amy, amylase; SP, starch phosphorylase; SPS, sucrose phosphate synthase; Inv, invertase; PFK, phosphofructokinase; PFP, pyrophosphate: fructose 6-phosphatephosphotransferase.



In potato tubers the starch-bound phosphate comprises a significant proportion of the total tuber phosphate content, but its function in starch metabolism is not clear. The same holds true for the biochemical reaction(s) leading to the formation of the starch phosphate monoesters (Ritte *et al.* 2001). A protein (designated as R1) has been recently identified using a proteomic approach, and circumstantial evidence suggests that it is involved in the phosphorylation of starch (Lorberth *et al.* 1998; Yu *et al.* 2001). The C-terminal sequence of the R1 protein shows some homology to bacterial PEP synthases (pyruvate, water dikinase EC 2.7.9.2), which transfer phosphate from ATP in a dikinase reaction to pyruvate and water. Antisense repression of R1 in potato tubers

leads to a strong reduction in the amount of starch-bound phosphate by 10-50% during storage at a low temperature (Lorberth *et al.* 1998).

Starch phosphorylase degrades starch by transferring a glycosyl residue from amylase onto inorganic phosphate to form glucose-1-phosphate (Sowokinos 1990). UTP α-D-glucose-1-phosphate uridylytransferase (UGPase) reversibly catalyzes the conversion between glucose-1-phosphate and UDP-glucose (Fig. 1.2), which is a rate-limiting substrate for sucrose synthesis. Environmental-induced expression, allelic variants, post-translational modifications, and substrate-induced kinetic changes regulate UGPase activity. Four weeks in cold storage increased expression of UGPase (Sowokinos 2001).

Once in the cytosol, these metabolites are converted to sucrose via sucrose phosphate synthase (SPS), which mediates the formation of sucrose-6-phosphate from UDP-glucose and fructose-6-phosphate (Krause *et al.* 1998). Sucrose-6-P phosphate phosphatase immediately dephosphorylates sucrose-6-phosphate to free sucrose. Subsequently, a proportion of the sucrose is irreversibly hydrolyzed to glucose and fructose by vacuolar acid invertase (Greiner *et al.* 1999).

Several isoforms of invertase with different biochemical properties and subcellular locations exist (Sturm 1999), but vacuolar soluble acid invertase transcription, activity, and inhibition best associate with the ratio of hexoses to sucrose. The presence of an endogenous inhibitor limits invertase activity in vitro (Pressey and Shawn 1966), but the inhibitor is located outside the vacuolar (Isla *et al.* 1992). Invertase activity of tubers increases in cold storage (McKenzie *et al.* 2005).

Several mechanisms have been advanced to explain the cold-induced accumulation of sugars in potatoes. An early proposal suggested that sweetening occurs because entry of hexose-P into glycolysis is restricted at low temperature, diverting the products of starch breakdown into the pathway of sucrose synthesis (ap Rees *et al.* 1981). Ap Rees *et al.* (1988) showed that the conversion of fructose 6-phosphate to fructose 1, 6-bisphosphate, the first committed step of glycolysis, is restricted in the cold. A second possibility is that sugar accumulation requires increased activity of the enzymes involved in sucrose synthesis. Although there is no consistent evidence for cold-induced changes in the maximum catalytic activity of SPS, accumulation of sucrose following transfer to the cold is associated with the appearance of a novel form of SPS (Reimholz *et al.* 1997), and with alterations in the kinetic properties of SPS likely to permit greater activity *in vivo* (Hill *et al.* 1996). A third possibility is that formation of glucose and fructose might be required to promote sugar accumulation by degrading sucrose under the activity of invertase. Some evidence has suggested that invertase determines the relative levels of sucrose and reducing sugars that accumulate rather than influencing the total amount of sugar that is formed during the cold storage (Richardson *et al.* 1990; Zrenner *et al.* 1996; Malone *et al.* 2006).

Selecting proper varieties for good chip quality

The major consideration in selecting varieties for the chipping industry is the ability of that variety to withstand unfavorable environmental and cultural factors and to maintain good chipping quality throughout the storage season (Work *et al.* 1981).

Quality traits important for evaluating cultivars used in potato chip color include high dry matter, low sugars, and freedom from defects (Dale and Mackay 1994). Chip defects can result from several external and internal problems including environmental stresses, pathogen infection, hollow heart, heat necrosis, mechanical injury, greening, and tuber rot. In addition to internal quality traits, chipping potatoes usually have an appearance typified by round shape and thin, smooth skin.

Potato breeders in North America have been very successful in improving chip quality in potatoes. This is evidenced by a slight improvement over time in cultivars for tuber solids, significant and large improvements for reducing sugar content and chip color, and a recent improvement in cultivars with respect to their ability to produce defect-free chips (Love *et al.* 1998). The rate of improvement for these traits does not appear to be diminishing. If current trends continue, breeders should expect additional improvement for chip color, reducing sugar contents, and defect-free chips (Love *et al.* 1998). Future trends in tuber solids are less predictable. Because higher solids are not consistently considered advantageous by the chipping industry, future changes may be erratic (Douches *et al.* 1996; Love *et al.* 1998).

Stem-end chip defect is a serious chip quality problem

Stem-end chip defect is a serious and costly issue for the potato chip industry in the U.S. (Bussan *et al.* 2009a). This defect is characterized by dark-colored vascular tissues and adjacent cortical tissues at the tuber stem end portion of potato chips after frying at high temperatures. Chips with stem-end chip defects are unappealing to consumers and

may cause raw product to be rejected at processing plants. Stem-end chip defect occurs erratically, with a high incidence in some years and low incidence in others. Defects were widespread across many U.S. production regions in 2006 and 2011, and prevalent in the upper Midwest in 2010. Few defects were observed in 2007-2009 (Wang *et al.* 2012).

Localized discoloration around tuber stem-end vascular tissues after frying for chips is the most typical characteristic of stem-end chip defect. As suggested by previous literature, dark color on the chip is likely caused by the Maillard reaction in which reducing sugars react with free amino acids. Thus factors that can induce localized accumulation of reducing sugars in stem-end tissues are potential causes of this problem.

The specific causes of stem-end chip defect are unknown. However, it is proposed that factors that cause this defect can be divided into two categories: the abiotic ones and biotic ones. Potential abiotic factors include water and/or heat stress during different tuber growth stages, chemical immaturity of tubers, as well as post harvest storage under inappropriate temperatures. The biotic factors include infection by pathogens, especially vascular pathogens such as *Verticillium* that may affect translocation during the growing season. Interactions between the abiotic and biotic factors cannot be neglected since they usually occur concurrently in the field.

What is most problematic is that stem-end discoloration can be missed by traditional sampling techniques used for quantifying sugar concentrations of chipping potatoes during chemical maturity monitoring and preconditioning of stored potatoes. As a result,

potato chips that should fry well based on sugar concentrations during sampling end up with dark fry color on the stem end (Bussan *et al.* 2009a).

Management practices that affect development of stem-end chip defect in chipping potatoes may include vine kill timing, vine kill product, nitrogen fertilizer rate and time of application, irrigation frequency, hill shape, heat stress, and other factors. Survey data from commercial potato fields has indicated that stem-end chip defect is not typically visible until late August or early September, and rarely visible until after vine kill. As a result, research needs to be focused on identification of methods for detecting early signs that will allow prediction of stem-end chip defect formation (Bussan *et al.* 2009a).

Goals of Research

Stem-end chip defect is an important tuber quality concern facing the chipping potato industry in the US. The losses due to this defect have not been quantified, but can be serious for growers because of crop rejection and for processors because of trim loss and product downgrades. Despite the importance of stem-end chip defect, there are few research reports about the causes of this important chipping potato quality issue. The primary goal of this research was to examine the influences of abiotic and biotic factors on incidence and severity of stem-end chip defect in tubers from climate-controlled greenhouse and from the field. Abiotic factors included moderate heat stress for two weeks (daytime temperature at 30°C), moderate water stress (soil water potential between -20 kpa to -80 kpa), stage of tuber bulking when the stresses

were imposed (early or late), tuber chemical maturity when harvested, as well as post harvest storage at 13°C for two months. Biotic factor was the infection of *Verticillium* wilt that occurred in non-fumigated relative to fumigated fields. The ultimate goal was to identify the causes of stem-end chip defect and to provide the chipping industry with suggestions on how to reduce both agricultural and economic losses due to this defect.

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Chapter 2: Stem-end chip defect in chipping potatoes (*Solanum tuberosum* L.) as influenced by mild environmental stresses

Global consumption of potato (*Solanum tuberosum* L.) continues to shift from fresh potatoes to value-added processed food products such as potato chips. One serious tuber quality defect of chipping potatoes is stem-end chip defect, which results in chips with dark-colored vasculature and adjacent tissues at the tuber stem end after frying. The influence of moderate water stress, alone or in combination with moderate heat stress, as well as maturity of tubers at harvest on the incidence and severity of stem-end chip defect was investigated. Treatments were imposed in controlled-environment greenhouses. Only temperature stress, daytime temperature at 30°C, in one of two years significantly changed the occurrence of stem-end chip defects. Biochemical analyses showed that more severe defects were associated with increased amounts of tuber stem-end glucose and increased stem-end acid invertase activity. We conclude that moderate environmental stresses and maturity of tubers at harvest are not sufficient to consistently cause stem-end chip defect.

Introduction

Processing potatoes must meet rigorous quality standards. Blemishes on chips or fries result in defects as they are undesirable to consumers and this may cause raw product to be rejected at processing facilities (Iritani 1981; Kleinkopf 1979; Shallenberger *et al.* 1959; Thompson *et al.* 2008). Stem-end chip defect is a serious quality concern for chipping potatoes that is characterized by dark-colored vasculature at the basal end, or stem end, of the tuber. A larger dark area predominantly inside of the vascular ring is often associated with the defect. Discoloration of vascular tissue alone does not typically lead to rejection of stem-end chip defect potatoes, but if the tuber tissue inside of the vascular ring fries dark, this can be rated as a defect (Bussan *et al.* 2009a).

Stem-end chip defect occurs erratically and with varying severity across years and locations. In some years, tubers with stem-end chip defect are widespread and common. In 2006 and 2011, stem-end chip defect was observed from Texas to the Upper Midwest. In 2010 it was prevalent in the Upper Midwest, but much less common elsewhere. Stem-end chip defects vary regionally year to year affecting between 5 and 25% of the crop on an annual basis (personal communication with USPB chip committee).

The localized nature of the defect, with predominant occurrence within 1 to 2 cm of the stem end of the potato, creates challenges in detecting the problem by current sampling techniques. Chip producers monitor tuber sugar concentrations of potatoes during late season growth to evaluate chemical maturity and identify optimal harvest time to maximize quality out of the field and in storage (Herrman *et al*. 1995; Sowokinos 1978).

Potatoes are also monitored during early storage to precondition tubers for optimal long-term storage (Bussan *et al.* 2009b; Glynn *et al.* 2004). Current methodology based on sampling and analysis of entire tubers may not detect potential for stem-end chip defects. Thus potato tubers that should fry well based on low sugar concentrations during sampling have dark color after frying on the stem end of chips and risk of rejection at the chipping plant (Bussan *et al.* 2009a).

Stem-end chip defect has similarities with sugar-end defect of fry processing potatoes, most notably, a localized dark fried color at the stem, but not bud end of the tuber. Sugar-end defect potatoes are typified by high amounts of reducing sugars at one end of the tuber (Thompson *et al.* 2008). The dark color at the stem end of sugar-end defect potatoes results from the Maillard reaction where reducing sugars reacting with free amino acids during frying to produce dark-colored pigments (Gould and Plimpton 1985; Shallenberger *et al.* 1959). Sugar-ends develop in response to stress such as moderate heat and drought stress at the early tuber bulking stage (Hiller and Thornton 1993; Iritani 1981, 1987; Iritani and Weller 1980; Kincaid *et al.* 1993; Kleinkopf 1979; Shock *et al.* 1992, 1993). Plant stress is also thought to be involved in stem-end chip defect formation, but there is little agreement as to the type of stress or the stage of crop growth that is most sensitive to development of the defect (Bethke *et al.* 2009). Another factor potentially contributing to the development of stem-end chip defect is the relative maturity of the potato tubers when harvested. Sowokinos (1978) demonstrated the influence of chemical immaturity on accumulation of sugars in tubers and formation of dark pigments in chips.

The erratic occurrence of stem-end chip defect in production fields and during post-harvest storage has made it difficult to conduct research on this important quality defect. Identifying environmental conditions or cultural management practices that cause defect formation would improve the ability to anticipate when defects are likely to occur, and select germplasm resistant to defect formation. These experiments were designed to test several putative causes of stem-end chip defect. Being able to create defects experimentally would enable more efficient research on defect development and changes in storage.

A two-year project was conducted in UW-Madison Biotron in 2008 and 2009 with the objective of documenting the influence of moderate water deficit, alone or in combination with elevated daytime temperature during early or late tuber bulking, maturity of tubers at harvest, and post-harvest storage of tubers on the incidence, severity and underlying biochemistry of stem-end chip defect.

Materials and methods

Plant Growth. Certified seed potatoes of cultivar FL1879 were cut and suberized prior to planting in 20 L pots filled with Metromix 366P soil (Sun Gro Horticulture, Bellevue, WA). FL1879 was chosen because it is a widely-grown chipping potato in which stem-end chip defects have been observed at harvest and in storage. Plants were grown in temperature-controlled greenhouses with 22°C/18°C day/night temperatures, with temperature increased at 05:00 AM and decreased at 21:00 PM. A minimum day length of 12 hours (06:00 AM to 18:00 PM) was provided by natural illumination supplemented

by sodium vapor lights as needed to keep photosynthetically active radiation above 500 μM m^{-2} s^{-1}. Plants were watered with two drip irrigation lines fed by time-activated pumps. One quarter-strength Hoagland`s solution was supplied for one to three minutes at 07:00 AM, 13:00 PM, and 18:00 PM under normal irrigation treatments. Watering-period duration was adjusted to accommodate plant growth and specific stress treatment. Soil water content for non-stressed controls was kept high by irrigating to field capacity at each watering. Pots were outfitted with wire mesh cages that forced plant growth upward and prevented tangling of vines.

Stress Treatments. Stress treatments included elevated daytime temperature and varying soil water availability at different potato growth stages. For each stress period, control and treated plants were moved to one of two additional greenhouses. Daytime temperatures were either 22°C from 05:00 AM to 21:00 PM or 30°C from 05:00 AM to 21:00 PM. The nighttime temperature for all treatments was 18°C. Plants were irrigated at two rates, non-stressed control and moderate water stress, corresponding to target soil water potentials of greater than -5 kPa and -20 kPa to -85 kPa. Soil moisture status in 1 or 2 representative pots was monitored every 15 minutes using Watermark model 200SS sensors (Irrometer, Riverside, CO) and TDR probes (Model CS616, Campbell Scientific, Logan, UT) attached to a Campbell Scientific CR10 datalogger with multiplexers (Campbell Scientific). These data were used to guide decisions on when to irrigate. Plants were watered lightly when soil water potentials fell below -60 kPa or when leaves began to show signs of wilting. Heterogeneity in soil moisture was observed as soils dried, and measured soil moisture contents were considered to be estimates of

soil moisture status. Soil temperature was monitored using buried thermocouples attached to the same dataloggers.

The morning watering on the day of harvest was omitted. At the end of each stress period, plants that were not harvested immediately were watered to field capacity and subsequent irrigation was identical to that for control plants. In 2008, stress periods occurred 36 to 50 days after planting (DAP, first stress period/early stress period), and 58 to 72 DAP (second stress period/late stress period). In 2009, stress periods occurred 45 to 59 DAP (first stress period), and 68 to 82 DAP (second stress period). Nine plants were treated with each combination of watering rate and temperature for both stress periods. After the second stress period, tuber maturity was varied by continuing to water some plants with one quarter-strength Hoagland`s solution to delay maturity while other plants were watered with reverse osmosis (RO) water to encourage maturation and vine senescence. Final harvest in 2008 was 154 DAP, and 156 DAP in 2009. Post-harvest sampling occurred two months after final harvest in both years. There were five total sampling time points in each year, first and second sampling occurred immediately after each stress period, a third harvest occurred when most leaves on plants watered with nutrient solution remained green while plants watered with RO water had extensive leaf and stem senescence (123 DAP in 2008 and 122 DAP in 2009), a fourth sampling period occurred when all plants had senesced naturally, and the last sampling was out of 13˚C storage two months later. Total nitrogen content of leaves from plants under both nutritional treatments prior to the third harvest was quantified by using the fourth fully expanded leaf from the top of the primary stem including the petiole.

Water Potential Measurements. At the end of the stress periods, plants were moved from greenhouses where treatments were implemented to an indoor work area prior to sampling. Water potential measurements of three leaves and three tubers per plant were obtained using a pressure chamber (Soil Moisture Corp, Santa Barbara, CA). Leaf and tuber samples were enclosed in plastic bags prior to cutting petioles or stolons in order to minimize evaporative water loss. Tuber water potentials were determined as described by Bland and Tanner (Bland and Tanner 1985), except that tubers were assayed immediately after removal from the plant instead of holding in plastic bags for 16 h prior to making measurements. Measurements were made between 09:00 AM and 12:00 PM. Tubers used for water potential measurements were placed in labeled mesh bags for subsequent sampling and analysis.

Sample Collection. Tissue samples for sugar determinations and acid invertase assays were removed from tubers within 24 h of harvest or removal from storage. During the first sampling, when tubers were at the early bulking stage, individual cores of tuber tissue 0.7 cm in diameter were removed with a cork borer from the center of tubers that had been used for water potential measurements. From the second to the last harvest, cores were taken as close as possible to the apical and basal ends of tubers. Cores were cut into 1 cm lengths that did not include periderm, weighed for fresh weight, placed into 1.5 ml conical tubes and frozen at -80°C.

Biochemical Analysis of Tuber Samples. Invertase assays and HPLC determination of tuber sugar contents were conducted as described in Bhaskar *et al.* (2010) except that 60 µl of protein extract was desalted on Zeba spin columns (Pierce Biotechnology,

Rockford, IL) rather than 1 ml of protein extract on PD-MidiTrap columns (GE Healthcare) .

Developing a Rating System for Stem-end chip defect. An index for rating the severity of stem-end chip defect in potato chips was developed (Fig. 2.1).

Fig. 2.1: Rating scale for stem-end chip defect.

In this system, no color development on the chip was a 0, dark color of vascular tissue only was a 1, color shadow surrounding the vascular tissue was a 2, color penetrating up to 1 cm into the tissue was a 3, color development up to 1.3 cm into the tuber tissue was a 4, and color development beyond 1.3 cm was a 5. Chips with scores of 0 to 2 are not scored as defect chips by processors. Chips with scores of 3 to 5 are scored as defect chips that may be unacceptable to chip processors because the defect extends 1 cm or more in from the edge of the tuber or chip. A total of 576 chips, each chip from the center of a single tuber and through the stem scar, three tubers from a plant, and three plants from a combination of treatments and harvest times in 2008 and 2009 were scored for stem-end chip defect based on the rating index.

Chip Frying and Scoring. Tubers were cut in half and a slice that was 1 mm in thickness

was cut off one half and discarded. A second slice was cut, rinsed in cold water and patted dry on terry towels. Chips were fried in cottonseed oil at 182°C for 2 minutes and 15 seconds. Chips were scored independently by two people according to the stem-end chip defect index, and scores of a single chip were averaged to balance the visual grading between the two people.

Data Analysis. For sugar data, Analysis of variance (ANOVA) was performed to test treatment effects by using the Linear Model (LM) procedure of R (R Development Core Team (2008)). Means separations were made using Fisher`s Least Significant Difference (LSD) at p=0.05. Main effects were sampling positions on the tuber, water stress, temperature stress, stress periods, tuber maturity at harvest, and different storage periods. Data from 2008 and 2009 were not combined due to heterogeneity of variances across years. Defect score data were analyzed to test treatment effects by using the Logit Model (GLM) procedure of R based on its binomial distribution. Differences of percentage of severe defects in each treatment were compared using Chi-square test. Main effects were water stress, temperature stress, stress periods, and tuber maturity at harvest. Data from 2008 and 2009 were not combined because year effect was significant.

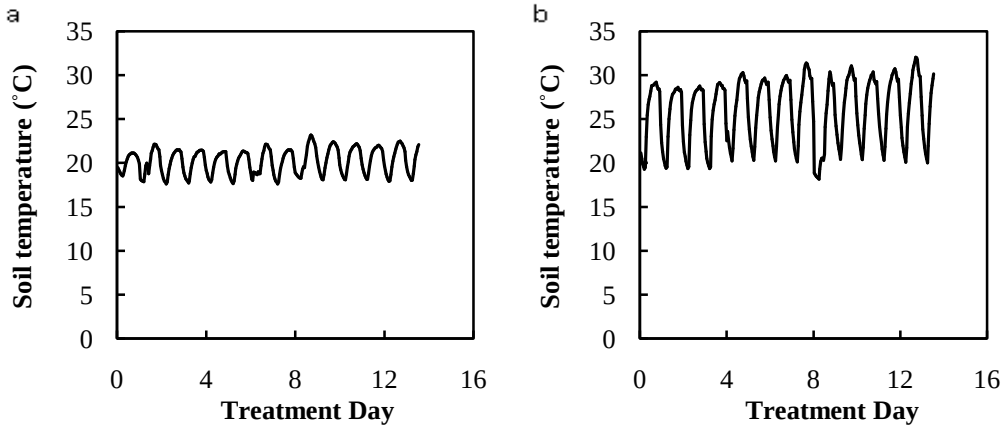
Results

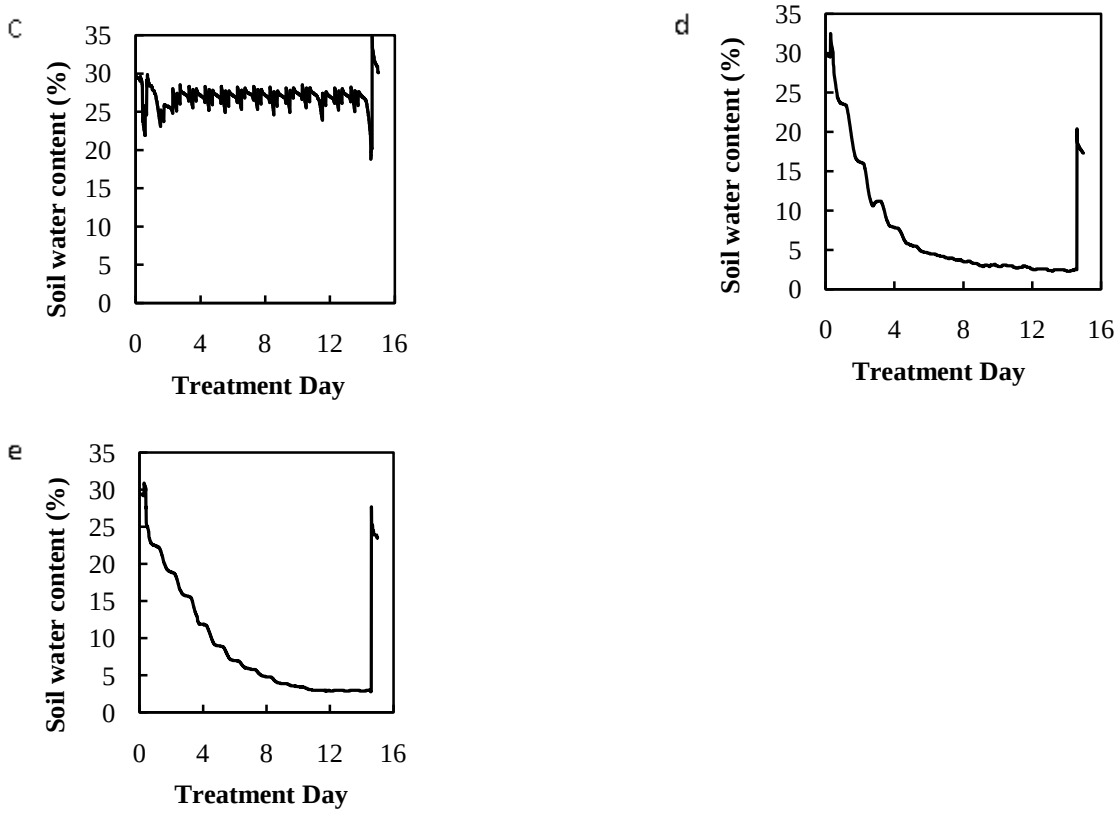
Responses of plants and tubers to moderate heat and water stress during growing season

Plants were exposed to moderate water stress and heat stress treatments during early

tuber bulking and late tuber bulking. Mean tuber size from control plants at the end of the early and late stress periods were 94 g and 312 g, respectively, in 2008 and 76 g and 257 g, respectively, in 2009. At daytime temperatures of 22˚C or 30˚C, soil temperature consistently tracked with the ambient air temperature (Figs. 2.2a, b). Soil water content of well-watered controls remained between 25% and 30% v/v through the 14 days of both stress periods (Fig. 2.2c). For the plants under water stress at daytime temperature of 22˚C, soil water content decreased from 30% to 3% v/v (Fig. 2.2d). At a daytime temperature of 30˚C soil water content decreased to approximately 3% v/v more rapidly than at 22˚C (Fig. 2.2e). Measured soil water potentials were high in well-watered pots, typically >-5 kPa, and were as low as -85 kPa in water-stress treatments when volumetric soil moisture content decreased to less than 5% (data not shown).

Fig. 2.2: Soil temperatures (a, b) and soil water content (c, d, e) during late tuber bulking for well-watered plants at daytime temperature of 22˚C (a, c) and for plants under moderate water deficit at daytime temperatures of 22˚C (d) or 30˚C (b, e) in 2009.





Mid-morning leaf and tuber water potentials measured at the end of each of the two stress periods reflected changes in the growth environment (Table 2.1). The combination of high daytime temperature, moderate soil water deficit and larger plant size at the late stress period resulted in the lowest water potentials observed in leaves and tubers in 2008 and 2009.

Table 2.1: Mid-morning tuber and leaf water potentials in 2008 and 2009. Plants were either well watered (W), or exposed to a period of moderate water stress (D) at daytime temperatures of 22˚C or 30˚C at early (Early) or late (Late) tuber bulking. Data points are mean water potentials +/- standard deviations for nine leaves and nine tubers from three invidual plants, with three leaves and three tubers from each plant.

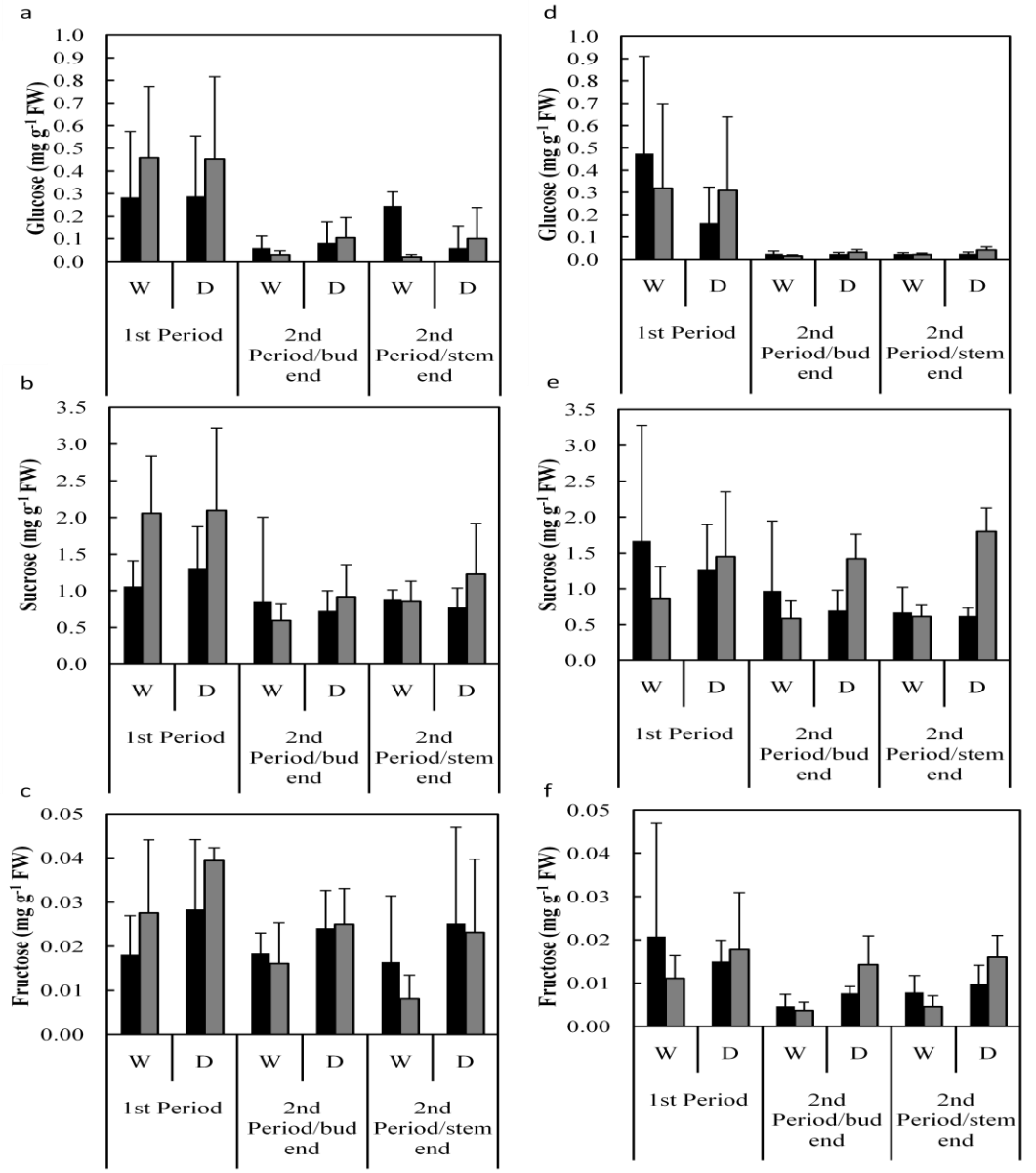
Treatment	Leaf (Mpa)	Tuber (Mpa)	Leaf (Mpa)	Tuber (Mpa)
	2008		2009	
Early 22˚ W	-0.14±0.05	-0.08±0.03	-0.20±0.03	-0.09±0.02
Early 30˚ W	-0.20±0.08	-0.12±0.04	-0.28±0.03	-0.18±0.04
Early 22˚ D	-0.19±0.06	-0.11±0.03	-0.23±0.07	-0.15±0.04
Early 30˚ D	-0.17±0.04	-0.13±0.02	-0.32±0.10	-0.24±0.03
Late 22˚ W	-0.13±0.06	-0.08±0.03	-0.14±0.10	-0.07±0.04
Late 30˚ W	-0.23±0.08	-0.12±0.06	-0.22±0.06	-0.10±0.04
Late 22˚ D	-0.16±0.05	-0.11±0.03	-0.16±0.03	-0.14±0.07
Late 30˚ D	-0.25±0.05	-0.28±0.10	-0.70±0.07	-0.61±0.06

Glucose contents across all treatments were higher in tubers harvested at the end of the early stress period than the late stress period in both years (Figs. 2.3a, d, $p<0.05$). Temperature treatment or water stress treatment did not affect mean tuber glucose content at early or late tuber bulking in 2009 (Fig. 2.3d). In 2008, the only treatment effect was found in the tubers under water sufficient condition at the late stress period that had greatly reduced glucose at daytime temperature of 30˚C (Fig. 2.3a, $p<0.05$). Tuber fructose contents were less than 0.04 mg g^{-1} FW in 2008 and 0.025 mg g^{-1} FW in 2009 for all treatments at early or late tuber bulking, and were not different between treatments (Figs. 2.3c, f).

Tuber sucrose content at the end of the second stress period was higher under water stress at a daytime temperature of 30˚C compared to 22˚C (Figs. 2.3b, e, $p<0.05$) in both 2008 and 2009. In contrast, tubers from well-watered controls tended to have less

sucrose at 30˚C compared with 22˚C (Figs. 2.3b, e) except for tubers from plants that did not receive a stress during the first stress period in 2008.

Fig. 2.3: Glucose (a, d), sucrose (b, e) and fructose (c, f) contents in tuber samples from well watered plants (W) and plants exposed to a period of moderate water stress (D) at daytime temperatures of 22˚ C (*black bars)* or 30˚C (*grey bars*). Each bar indicates the mean standard deviation for nine samples, with each sample made up of tuber tissues from one individual tuber at the end of the early or late stress periods in 2008 (a, b, c) and 2009 (d, e, f), respectively.



Plant and tuber responses to nutrient availability

The extent of vine senescence at the third harvest (Fig. 2.4) had a significant effect on tuber bud-end and stem-end sucrose content, indicating different extents of tuber maturity (Table 2.2).

Fig. 2.4: Potato vines showing varying maturity as a result of continued irrigation with nutrient solution (left two plants) or irrigation with RO water (right two plants).

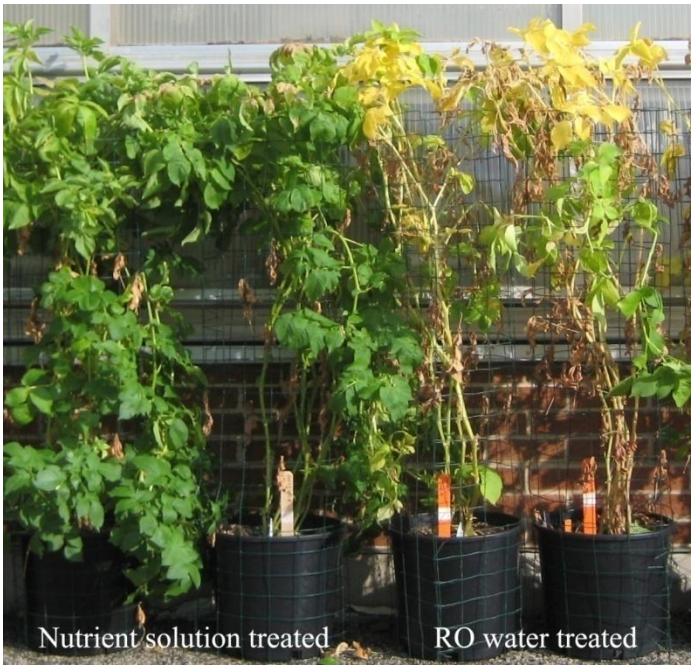


Table 2.2: Sucrose and glucose content (mg g^{-1} FW) in the stem end or bud end of tubers from plants treated with reverse osmosis (RO) water or nutrient solution to delay maturity in 2008 and 2009. Sugar contents are means ± standard deviations. Within each column, numbers followed by the same letter do not differ significantly using LSD at p=0.05.

Year	2008		2009	
Nutrient treatment/ Sampling position	Sucrose	Glucose	Sucrose	Glucose
RO Water/Stem	0.71±0.18a	0.07±0.04a	0.93±0.19a	0.06±0.07a
Nutrient Solution/Stem	0.94±0.32b	0.06±0.04a	1.08±0.35b	0.03±0.03b
RO Water/Bud	0.75±0.35a	0.16±0.04b	0.84±0.26a	0.06±0.09a
Nutrient Solution/Bud	1.02±0.45c	0.14±0.03b	0.94±0.24a	0.05±0.03a

Mean sucrose was greater (p<0.05) at both ends of tubers harvested from plants with green vines rather than plants with senesced vines when averaged across all treatments and sampling periods (Table 2.2). Glucose content at bud end and stem end of tubers from plants watered with RO water were higher than those in tubers from plants watered with nutrient solution (Table 2.2, p<0.05 at the stem end). Sucrose and glucose contents of tubers from the various environmental stress or tuber maturity treatments in 2008 and 2009 were not different between plants irrigated with RO water and plants irrigated with nutrient-solution to delay vine senescence. Total nitrogen content of leaves from plants irrigated with nutrient solution averaged 3.02$\pm$0.06% in 2008 and 2.44$\pm$0.09% in 2009 on a dry weight basis, whereas those on plants irrigated with RO water had a nitrogen content of 1.89$\pm$0.03% in 2008 and 1.64$\pm$0.01% in 2009.

Moderate environmental stress and tuber maturity had little effect on stem-end chip defect

In the 576 chips that were fried and scored over two years, 17% had severe stem-end

chip defect scores of 3, 4 and 5, 55% had mild stem-end chip defect scores of 1 and 2, and 28% did not have stem-end discoloration and were given a score of 0. Defects with scores of 4 or 5 were rarely found in either year and occurred in less than 6% of all chips. Water deficit, stresses at different growing stages, as well as maturity of tubers at harvest did not affect incidence of severe defects in both years (Table 2.3). The only effect was heat stress of 30˚C that led to 14% severe stem-end chip defects compared to 6% for control plants at 22˚C in 2009 (Table 2.3, $p<0.05$).

Table 2.3: The percentage of chips with defect score of 3, 4, or 5 (severe defect) are indicated for 2008 and 2009. Within each year, values followed by the same letter in each treatment do not differ significantly using Chi-square test at $p=0.05$.

Year		2008	2009
Treatment	**Controls vs Stresses**	**Percentage of chips with severe defect**	**Percentage of chips with severe defect**
Water	**Well watered**	22a	8a
	Water stressed	25a	12a
Temperature	**22°C**	23a	6a
	30°C	26a	14b
Stress Period	**Early tuber bulking**	26a	12a
	Late tuber bulking	22a	8a
Maturity at harvest	**Mature**	26a	10a
	Immature	22a	10a

Post harvest tuber sugar contents and defect incidence in chips

Tubers stored at 13˚C for two months experienced changes in sucrose and glucose contents at both ends of the tuber. Sucrose decreased and glucose increased at the bud and stem ends of the tuber in both years (Table 2.4, $p<0.05$). Despite this, the percentage of chips with defect scores of 3, 4 or 5 only changed by 2% during storage in

both years (Table 2.5). The percentage of chips without a defect, however, increased during storage from 17% to 22% in 2008 and 27% to 49% in 2009 (Table 2.5, $p<0.05$).

Table 2.4: Sucrose and glucose content (mg g^{-1} FW) in the stem end or bud end of tubers across temperature, moisture and vine maturity treatments before and after two months of storage at 13˚C in 2008 and 2009. Sugar contents are means ± standard deviations. Within each column, sucrose contents or glucose contents followed by the same letter do not differ significantly using LSD at the $p=0.05$.

Year	2008		2009	
Storage Period/ Sampling Position	**Sucrose**	**Glucose**	**Sucrose**	**Glucose**
Before storage/Stem	0.93±0.24a	0.09±0.04a	1.00±0.33a	0.07±0.03a
After storage/Stem	0.72±0.22b	0.18±0.12b	0.95±0.29b	0.07±0.04a
Before storage/Bud	0.99±0.32a	0.04±0.02c	0.99±0.26b	0.04±0.01b
After storage/Bud	0.76±0.17b	0.08±0.03a	0.74±0.15c	0.06±0.03a

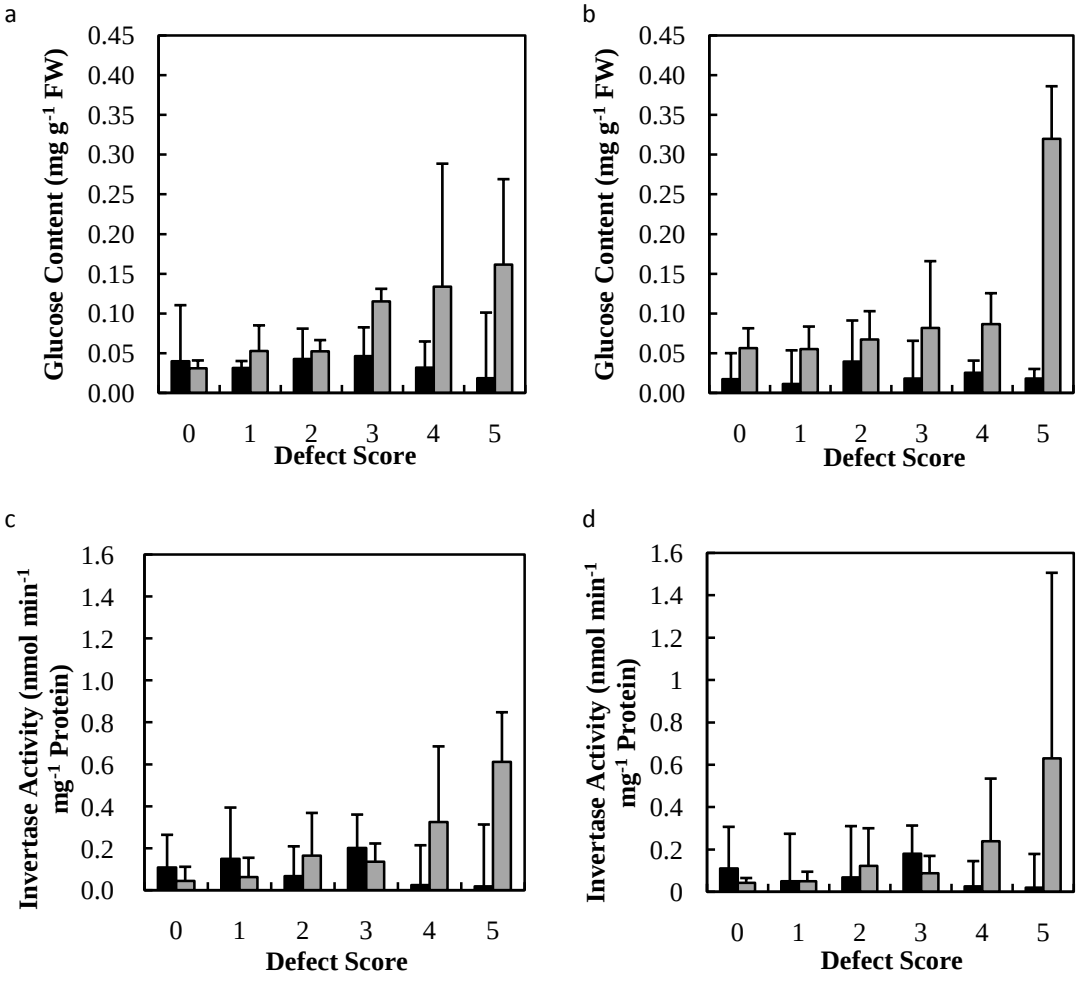
Table 2.5: Stem-end chip defect percentage in tubers before and after two months storage at 13˚C in year of 2008 and 2009. Within each column, values followed by the same letter do not differ significantly after Chi-square test at $p=0.05$.

Year	2008			2009		
	Severe defects with scores of 3, 4 or 5 (%)	**Mild defects with scores of 1 or 2 (%)**	**Defect free with score 0 (%)**	**Severe defects with scores of 3, 4 or 5 (%)**	**Mild defects with scores of 1 or 2 (%)**	**Defect free with score 0 (%)**
Before storage	23a	58a	17a	9a	64a	27a
After storage	25a	55a	22b	11a	40b	49b

Glucose content and acid invertase activity at the tuber stem end and bud end of stem-end chip defect tubers

The glucose content and activity of acid invertase at the stem and bud ends of tubers across the full range of defect scores were measured. Samples from 2008 and 2009 were analyzed together since there was no difference between years when tubers with the same defect score were compared (p>0.05). At the tuber stem end, an association was observed between defect score and glucose content (Figs. 2.5a, b), as well as between defect score and acid invertase activity (Figs. 2.5c, d). Higher glucose content and invertase activities were observed in tubers with higher defect scores. Variability in acid invertase activity was large, perhaps because of the localized, non-uniform nature of the defect within tuber tissues and the small size of the tissue sample analyzed. Differences in glucose content and invertase activity between freshly harvested and stored tubers were not observed when comparisons were made between samples with the same defect score. Glucose content and acid invertase activity at the bud end of the same tubers did not correlate with stem-end chip defect score (Fig. 2.5).

Fig. 2.5: Glucose content (a, b) and acid invertase activity (c, d) on the stem end (*black bars*) and bud end (*gray bars*) of tubers with a range of stem-end chip defect scores at harvest (a, c) or out of storage (b, d). Each bar indicates the mean standard deviation of samples.



Discussion

We hypothesized that the causes of stem-end chip defect might be similar to those that cause sugar-end defect and dark fried color in processing potatoes such as Russet Burbank. Although there are common features between stem-end chip defect formation and sugar-end defect formation, several differences were observed between these two

important quality defects. For example, the dark color observed in chips from stem-end chip defect tubers is associated with the vasculature, and this is not a common feature of sugar-end tubers. Moderate water stress was not sufficient to cause stem-end chip defects (Table 2.3), but can cause sugar-end defects in processing potatoes (Eldredge *et al.* 1996; Shock *et al.* 1992, 1993, 1998). A relationship between tuber bud or stem-end sucrose and stem-end chip defect severity was not observed. This is in contrast to a previous report where sucrose was reduced at the tuber stem end in sugar-end tissues (Sowokinos *et al.* 2000). Similarly, extensive preconditioning of sugar-end defect tubers does not remove the defect (Glynn *et al.* 2004), but preconditioning of stem-end chip defect tubers can remove the defect in some cultivars based on preliminary data. In stem-end chip defect tubers, as in sugar-end defect tubers, glucose content and acid invertase activity are elevated at the tuber stem end (Fig. 2.5, Thompson *et al*. 2008).

The data presented here show that the applied stress treatments, including heat or water stress, as well as varied tuber maturity at harvest, resulted in physical and biochemical changes that are consistent with prior observations. Leaf and tuber water potentials decreased during water stress (Table 2.1).

Tuber sucrose was higher in tubers harvested at early tuber bulking and lower at late tuber bulking (Figs. 2.3b, e), higher when tubers were harvested while vines were green rather than after complete vine senescence (Table 2.2) and decreased in tubers during storage, with a larger decrease observed at the tuber bud end than at the tuber stem end (Table 2.4). Moderate water and heat stress during early or late tuber bulking increased tuber sucrose relative to non-stressed controls, as was shown previously for

Russet Burbank (Bethke *et al*. 2009). Unlike Russet Burbank, this effect was most apparent at the tuber stem end (Figs. 2.3b, e). As expected, tuber glucose contents were very high at early tuber bulking and much lower at late tuber bulking and at harvest (Figs. 2.3a, d). Most of the applied treatments, however, were not sufficient to cause an increase in either the incidence or severity of stem-end chip defects. The only exception to this was for temperature in 2009, where the percentage of severe defects increased from 6% to 14% when plants were treated with 30˚C daytime temperature (Table 2.3), but this difference of 8% is still relatively small.

Based on data presented here, two hypotheses for the formation of stem-end chip defect are proposed. First, defect formation may require a more extreme environmental stress, such as higher daytime or nighttime temperatures, greater soil moisture deficits, or stress periods longer than 14 days. Given that stem-end chip defects are observed in well-managed, irrigated production fields, it is unlikely that severe, extended water deficits are a possible cause of defect formation. High temperatures are unavoidable and like stem-end chip defects are more common in some years than in others. This observation along with the data in Table 2.2 showing that moderately high daytime temperature increased the percentage of severe stem-end chip defects suggests that further information is needed about temperature influences on stem-end chip defect formation. The second hypothesis for stem-end chip defect formation is that it is not strictly a physiological defect like sugar-end defect, but is in part a response to infection of the plant by a pathogen. Although the experiments reported here were conducted in greenhouses rather than production fields, we cannot exclude the possibility that

pathogens found on seed pieces, in soil or vectored by insects may have played a role in defect formation. It seems unlikely that tuber maturity on its own plays a major role in defect formation, given that defect rates at harvest and out of storage were comparable regardless of when tubers were harvested, including at early or late tuber bulking, late in the season when plants retained green leaves, and after vines had senesced completely (Tables 2.4 and 2.5).

Tubers with varying severity of stem-end chip defect were used for biochemical analyses. It was hypothesized that the dark color of stem-end chip defect is caused by the Maillard reaction in which reducing sugars react with free amino acids during frying, and that reducing sugars are produced when sucrose is cleaved by acid invertase. Elevated glucose contents were observed at the stem end of those tubers with more severe stem-end chip defects (Figs. 2.5a, b). Higher acid invertase activities were also observed in tubers that had the most severe stem-end chip defects (Figs. 2.5c, d). Glucose and acid invertase activity did not vary with defect severity in bud-end samples from the same tubers (Fig. 2.5). These data suggest strongly that acid invertase activity is up-regulated at the tuber stem end, and these higher activities give rise to more severe defects through the production of reducing sugars. This is similar to the case for sugar-end defects in russet or elongated tubers (Sowokinos *et al.* 2000). The molecular signals that result in acid invertase activation have not been described for sugar-end defect tubers, and one can only speculate as to whether they are the same in stem-end chip defect tubers. The region of high acid invertase activity in stem-end chip defect tubers is likely to be more localized than it is in sugar-end defect tubers, except perhaps

in the case of so called “sugar tips”, where only the extreme part of the stem end of processing russets fries dark. Sampling procedures for identifying or monitoring changes in sugar content associated with stem-end chip defect need to account for the highly localized nature of the defect. Whole tuber samples are likely to be inappropriate since the mass of tuber with the defect is much less than the mass of the tuber as a whole.

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Chapter 3: Effects of *Verticillium dahliae* infection on stem-end chip defect development in potatoes (*Solanum tuberosum* L.)

Potato chips are America's favorite snack food with annual retail sales of over $6 billion (Snack Food Association 2003). Stem-end chip defect, which is characterized by discoloration of the vasculature and surrounding tissues at the tuber stem end portion of chips, is an important tuber quality concern for U.S. chip production. The cause of stem-end chip defect is not known. *Verticillium* wilt, caused by a vascular fungal pathogen *Verticillium dahliae,* is a persistent potato disease that causes early plant senescence and yield reductions. A two-year field trial was conducted to investigate the effects of *V. dahliae* on stem-end chip defect development, tuber sugar contents, and the activity of acid invertase at the basal (stem) and apical (bud) ends of the tuber. Our results show that potato plants that were more infected with *V. dahliae* had a higher incidence of severe stem-end chip defects than plants with less *V. dahliae* infection. *V. dahliae* infection appears to induce the up-regulation of acid invertase activity in tubers resulting in accumulation of reducing sugars, which are the direct cause of dark color on chips due to Maillard reaction.

Introduction

Stem-end chip defect is a serious quality concern for the potato chip industry. Chips with this defect are unacceptably dark along the vasculature and adjacent tissues at a position corresponding with the tuber stem end. Stem-end chip defects occur erratically between years and locations, with defects appearing locally in some years, and regionally in others (Wang *et al.* 2011). Defects are unsightly and contribute disproportionately to chip acrylamide content (Wang *et al.* 2012), which is currently an important issue for food safety and human health. Tubers that produce stem-end chip defect chips are undesirable to consumers, increase financial risks to chip growers, and can cause supply problems and quality concerns for processors.

Previous data have shown that stem-end chip defects are associated with high reducing sugar contents and elevated acid invertase activities on the tuber stem end, but not the tuber bud end. Reducing sugars react with free amino acids in the Maillard reaction when tuber slices are fried in hot oil for chips (Sowokinos *et al.* 1990) and thus elevated reducing sugars are the immediate cause of dark color at the chip stem end.

Based on preliminary data, we proposed that stem-end chip defect may result from abiotic stresses, biotic stresses, or a combination of the two. The abiotic factors may include a short period of heat stress during growing season (Wang *et al.* 2012). Biotic factors may include pathogens, with pathogens such as *Verticillium dahliae* that infect the vasculature of potato plants being obvious candidates for detailed investigation. Interactions between abiotic and biotic factors should also be considered since they

often happen concurrently in the field.

Verticillium dahliae is a soil-borne vascular fungal pathogen that can lead to yield losses of 10-50% and decreases in crop quality in potatoes (Powelson and Rowe 1993) and other crops. The disease *V. dahliae* causes is variously called *Verticillium* wilt (Vw), potato early dying (PED), or vascular wilt (Fradin *et al.* 2009). Vw is a persistent and serious problem in potato production (Jansky 2009), distributed throughout potato growing regions of the world (Powelson and Rowe 1993). Two species of *Verticillium* can cause Vw, *V. dahliae* Kleb and *V. albo-atrum* Reinke and Berthold. *V. dahliae* is more widespread in the US, especially in production areas where average daily summer temperatures commonly exceed 25°C (Johnson and Dung 2010).

Treating Vw is particularly difficult since many fungicides cannot reach vascular pathogens, and few fungicides exist to cure plants once they are infected (Fradin *et al.* 2009). Because microsclerotia of *V. dahliae* can survive in the soil for over ten years (Griffiths 1970), the effectiveness of cultural practices that can reduce the severity of Vw is variable and sometimes low compared with the economic return associated with chemical control by soil fumigation (Frost and Rouse 2006). Soil fumigation is currently the most effective approach to control the Vw disease. It is used extensively in many production areas with good success. However, this practice is expensive and application protocols must be followed carefully in order to avoid human health and environmental problems (Rowe *et al.* 1987; Fradin and Thomma 2006). Biological control has potential, but consistent results have not yet been obtained (Entry *et al.* 2000; Nagtzaam *et al.* 1998; Sessitsch *et al.* 2004).

A two-year research project was conducted in UW-Madison Hancock Agricultural Research Station in 2010 and 2011 to test the hypothesis that *V. dahliae* contributes to the formation and development of stem-end chip defect in chipping potato cultivars.

Materials and methods

2010 and 2011 field season at Hancock. Research was conducted at the University of Wisconsin Hancock Agricultural Research Station. The non-fumigated, *Verticillium dahliae*-infested field at this location has been planted with potatoes continuously for about six years and contained approximately 6.5 CFU of *V. dahliae* per gram of soil in 2010. Fields with reduced disease pressure were created by fumigating with Vapam HL (AMVAC Chemical Corporation, Newport Beach, CA) at a rate of 40 gallons/acre applied by shank injection the previous fall. On May 4th, 2010, and May 2nd, 2011, FL1879, FL2053, and Snowden, were planted by hand in the two trial fields with three replicates in each field. Each replicate was a single block in 2010 with ten seed tubers planted two feet apart whereas in 2011 each replicate was two blocks containing five seed tubers planted one foot apart. Potato plots were maintained using best management practices based on University of Wisconsin-Extension recommendations.

Visual ratings. Field plots were scored for Vw based on the percent chlorotic, necrotic, or wilted foliage on 26th July, 12th, and 20th August in 2010, and on 27th July, 16th and 29th August in 2011. Length of primary stems of each plant was measured on 26th July in 2010 and 27th July in 2011 as an indicator of canopy development in the two fields.

Field samplings. The first two tuber harvests occurred on 28th July and 16th August in

2010, and 28th July and 17th August in 2011, respectively. Reglone (Syngenta, Cambridge, UK) was applied 10 days before final harvest in 2010 and 14 days before final harvest in 2011 to desiccate vines that remained green. A third harvest (final harvest) was conducted when all plants had completely senesced, on 30th August in 2010 and 13th September in 2011. At the second and final harvest, number of tubers produced by each plant was counted. Tuber yields from each plant were determined by weighing at the final harvest. During each sampling, ten hills of every variety were harvested by hand and identified by hill. Tubers were placed into mesh bags and half of them were processed one day later. The other half was immediately moved to storage at 13°C and stored for two months, after which a fourth sampling occurred. At the first two harvests, main stems from each hill were collected and bulked for stem sap assay. At the final harvest, main stems that were wilted and dried were cut and bulked from each hill for dry stem assay.

***Verticillium* culturing assays.** *Stem sap assay.* A 10-cm section of each main stem was cut 8 to 10 cm above the soil surface. The bulked stems were rinsed in tap water, surface disinfested in 1% bleach for one minute, rinsed in sterile distilled water, and then wrapped in a 20×20 cm plastic bag. Each bulked stem sample was squeezed through a press to extract sap and a 100-µl aliquot was plated onto NPX medium (Butterfield and DeVay, 1977). Fungal colonies were counted using a stereomicroscope 14 days later after growth at ambient temperature without light. *Dry stem assay.* For each bulked dry stem sample, the apical 10-cm portion was placed in a paper bag and allowed to air dry for two months. Leaves were removed from the dried stem apices and stems were ground

in a Wiley mill with a 40-mesh screen. Per sample, 50 mg was plated on NPX medium. Fungal colonies were allowed to grow for 14 days at ambient temperature without light. Plant debris was washed from the plate by gently rubbing the surface of the agar medium with fingers under a stream of tap water. Fungal colonies on each plate were counted using a stereomicroscope.

Lab samplings. Tissue samples for sugar determinations and acid invertase assays were conducted as described in Bethke *et al.* (2009) with tissue cores (0.7 mm diameter) removed at each sampling time from positions as close as possible to the vasculature at the apical (bud) and basal (stem) ends of tubers.

Biochemical analysis of tuber tissue samples. HPLC determination of tuber sugar contents were performed according to published protocols in Bethke *et al.* (2009). FL1879 was selected for invertase assays because all stem-end chip defect scores were sufficiently well represented for that variety. For invertase activity assays, frozen tissue samples of 0.50-0.75g were ground in a freezer mill (SPEX SamplePrep, Metuchen, NJ) and stored at -80°C until extracted for proteins. Powdered tissue samples were mixed with 2 ml extraction buffer and 4-5 small glass beads. The extraction buffer contains 50 mM HEPES-KOH, pH 7.5, 5 mM MgCl2•6H2O, 1 mM EDTA, 1 mM EGTA, 0.1% w/v Triton X-100, 10% w/v Glycerol, 5 mM DTT and 1 mM PMSF. Homogenized extracts were incubated on ice and shaken every few minutes by hand. Homogenate were then centrifuged 10 min at 4°C at 16,000×rpm in a tabletop microfuge to pellet debris. Supernatant of 1 ml were desalted using PD MidiTrap G25 columns following the manufacture`s directions for gravity flow (GE Healthcare, Piscataway, NJ). About 250

microliters of desalted extract were placed into 0.2 ml strip-tubes and shaken using a Vortex Genie-2 (Scientific Industries) at speed 6 for 30 min at 4˚C to minimize the activity of invertase inhibitor proteins. Enzyme assays contained 20µl protein extract and 60µl of reaction buffer containing 133 mM sucrose and 26.7 mM Na-Acetate pH 4.7 and were incubated at 30°C. After 60 min 8µl Na-Phosphate (1M, pH 7.4) was added to stop the reaction and tubes were heated at 97°C for 3 min to inactivate the enzyme. Control samples contained all additions but were neutralized and heated immediately. Net glucose formed was quantified using the assay described in Bethke and Busse (2008). Protein content of desalted extracts was measured using the Bio-Rad protein quantification reagent (Bio-Rad Laboratories, Hercules, CA) following the manufacture`s directions for the microassay. Invertase activity was calculated as nmol glucose formed per min per mg of proteins.

Chip frying and scoring. Longitudinal slices were cut through the stem end attachment point through the center of each tuber and were fried and rated for stem-end chip defect on a scale of 0-5 according to index in Bussan *et al.* (2009a). Since chips with sores of 3, 4 and 5 maybe scored as defects at the processing facility, those three scores were categorized as severe defect. On the contrary, chips with scores of 0, 1 and 2 are likely meet processor requirements for defect free.

Data analysis. For data of primary stem length, number of tubers produced in each hill, tuber yields in each hill, as well as log-transformed CFU values, student`s t-test was conducted to test the pathogen treatment effect within each variety. Data from 2010 and 2011 were combined since there were no significant year effects. Defect score data

were analyzed to test pathogen treatment effects at each sampling time point by using the Logit Model (GLM) procedure of R (R Development Core Team (2008)) based on its binomial distribution. Differences of percentage of severe defects under each treatment for each variety were compared using Chi-square test. Data from 2010 and 2011 were combined because year effect was not significant. For sugar data, analysis of variance (ANOVA) was performed to test treatment effects by using the Linear Model (LM) procedure of R. Means separations were made using Fisher`s Least Significant Difference (LSD) at p=0.05. Main effects were years, varieties, sampling positions on the tuber, different pathological treatments, and different storage periods. Data from 2010 and 2011 are presented separately due to significant interactions between year and sampling position on the tuber. Pathogen and storage treatment effects on invertase activities were analyzed using student`s t-test in R.

Results

Increased V. dahliae pressure was associated with increased incidence of severe stem-end chip defects

In field year 2010, none of the plants in the fumigated field showed symptoms of *Verticillium* wilt. In comparison, plants in the non-fumigated field showed progressive development of Vw disease symptoms from the early season to final harvest (Table 3.1). In field year 2011, the plants in the fumigated field showed signs of Vw disease pressure, and different varieties showed different levels. FL1879 displayed the highest percentage

of plants showing Vw disease symptoms (data not shown). The non-fumigated field had more plants with Vw disease symptoms than the fumigated field (Table 3.1).

Table 3.1: Percentage of plants across the three varieties showing *Verticillium* wilt symptoms during the season.

Year	2010		2011	
	Fumigated	**Non-fumigated**	**Fumigated**	**Non-fumigated**
Early Season	0%	5-10%	0%	5-20%
Late Season	0%	40-50%	10-60%	60-90%
Final Harvest	0%	70-85%	10-80%	90-100%

Few or no severe defects were found at the early tuber bulking stage in either the fumigated or non-fumigated field (Table 3.2). During the late tuber bulking stage, however, stem-end chip defects were observed in each of the three varieties examined, with FL1879 having a severe defect percentage of 40% when grown under conditions of high infection pressure, compared with only 4% when grown with less infection pressure ($p<0.05$). At late tuber bulking, FL2053 and Snowden did not display significantly higher severe stem-end chip defect percentages when grown under non-fumigated rather than to fumigated conditions.

At final harvest, FL1879 had a 17% greater severe defect percentage in tubers from the non-fumigated field than in those from fumigated field ($p<0.05$). FL2053 at this time also displayed significantly higher severe stem-end chip defect incidence in the field with higher pathogen pressure. Stem-end chip defects were not significantly different

between fumigated and non-fumigated fields for Snowden at harvest.

After two months of storage at 13°C, tubers from plants exposed to greater *V. dahliae* pressure tended to produced chips with more severe defects than control tubers, and this difference was statistically significant at p=0.05 for FL1879.

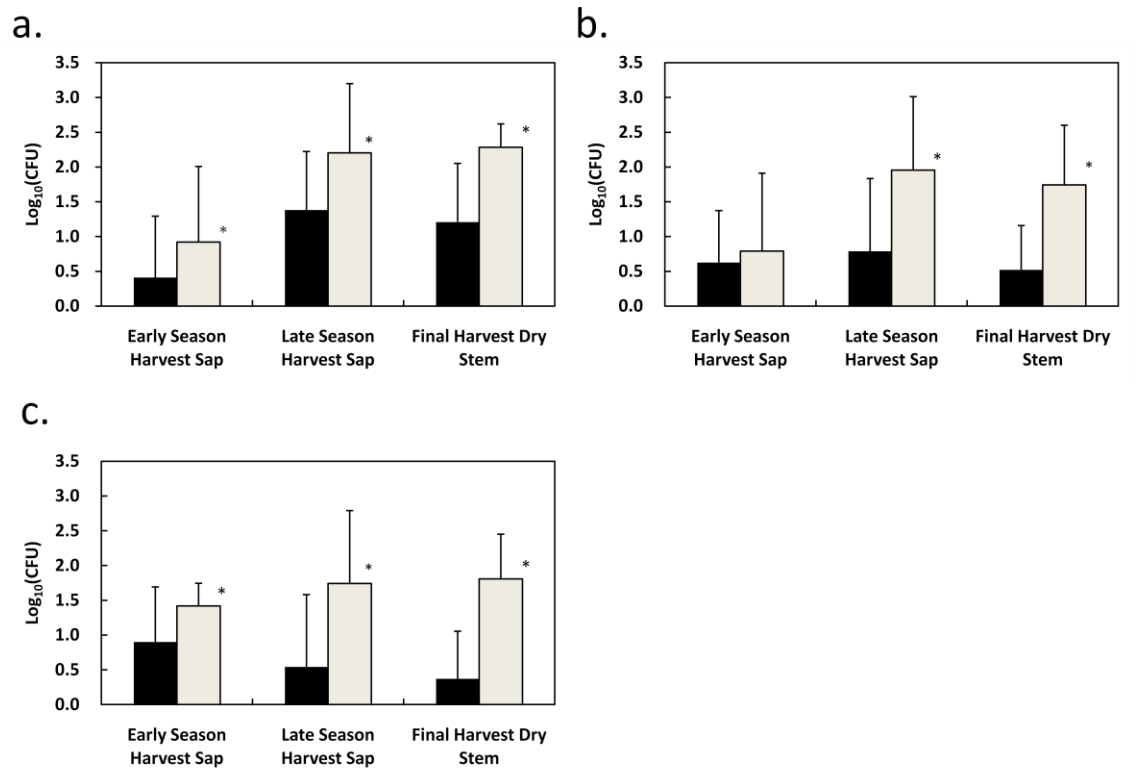
Table 3.2: Percentage of chips with severe stem-end chip defects in each variety under growth in fumigated (C) or non-fumigated conditions (V) at four different sampling time points. At each time point for each variety, values followed by the same letter did not differ significantly between fumigation treatments using Chi-square test at p=0.05.

Variety	Treatment	Early Season Harvest	Late Season Harvest	Final harvest	Out of storage Sampling
FL1879	**C**	0a	4a	28a	36a
	V	0a	40b	45b	59b
FL2053	**C**	0a	0a	12a	18a
	V	0a	3a	22b	25a
Snowden	**C**	0a	2a	22a	23a
	V	2a	9a	30a	28a

Plants in the non-fumigated field had higher rates of V. dahliae infection than those in the fumigated field

At early tuber bulking, sap from freshly cut primary stems had higher CFU from the non-fumigated field than from the fumigated field across all varieties (Fig. 3.1). During the late season, all varieties showed higher sap CFU in stems from the non-fumigated field. All three varieties had greater CFU counts in dry stem from the non-fumigated relative to the fumigated field (p<0.05).

Fig 3.1: Mean log10-tranformed Colony Form Units (CFU) in stems of potato plants grown in fumigated (*black bars*) or non-fumigated soil (*grey bars*) at early season, late season and final harvest for FL1879 (a), FL2053 (b), and Snowden (c). Error bars are standard deviations of 20 plants. An asterisk symbol was used to indicate the statistically significant difference between the two treatments at each sampling time point.



Greater infection with *V. dahliae* greatly affected crop growth. Primary stems from each plant in the field with lower Vw pressure were significantly longer than those in the field with higher Vw pressure for all the varieties (Table 3.3, $p<0.05$), and tuber number per hill was often less (Table 3.3). Total tuber yields were significantly reduced in the plants with the highest rates of fungal infection compared with control plants across all the varieties (Table 3.3, $p<0.05$).

Table 3.3: Average length of primary stems at the early season harvest, number of tubers produced in each plant at the late season harvest and final harvest, tuber yields in each plant at final harvest of each variety in fumigated field (C) or non-fumigated fields (V). For each variety, values followed by different letters significantly differ from each other by Student`s t-test at p=0.05.

Variety	Treatment	Average length of primary stems per plants in season (cm)	Number of tubers produced per plant in season	Number of tubers produced per plant at harvest	Tuber yield per plant at harvest (kg)
FL1879	**C**	81±12a	17±5a	15±4a	3.1±1.0a
	V	60±8b	11±4b	12±6a	2.2±1.3b
FL2053	**C**	85±12a	19±6a	16±7a	2.4±1.0a
	V	65±11b	15±5b	14±4a	1.8±0.4b
Snowden	**C**	104±18a	16±4a	19±6a	3.0±1.1a
	V	73±9b	17±4a	15±5b	2.4±0.9b

Tuber sucrose and glucose contents were affected by V. dahliae infection

Sucrose contents at the tuber stem end in all three varieties were higher at harvest in the more highly *V. dahliae* stressed plants than the controls in both years (Table 3.4a, b, p<0.05). Likewise, plants from the non-fumigated field tended to have greater amounts of sucrose on the bud end of tubers than those from fumigated fields at harvest in both years (Table 3.4a, b). Regardless of pathogen pressure or position on the tuber, sucrose contents decreased during two months of storage at 13˚C. Glucose contents on the stem end of tubers in all the three varieties were greater after two months of storage than at harvest in 2010 and 2011 (Table 3.5a, b, p<0.05). In 2010 for FL1879, bud-end sucrose was lower than stem-end sucrose in both treatments and both storage time points. However, FL2053 and Snowden showed the opposite bud-end and stem-end sucrose comparisons (Table 3.4a). In 2011, all the three varieties had higher stem-end sucrose

compared with bud-end sucrose (Table 3.4b). Glucose on the stem end of tubers was always higher than on the bud end in both years (Table 3.5a, b).

Table 3.4a: Bud-end and stem-end sucrose content (mg g^{-1} FW) of tubers from fumigated field (C) or non-fumigated field (V) before and after post harvest storage at 13°C for two months in 2010. Within each column, values followed by the same letter did not differ significantly using LSD at p=0.05.

Location	Treatment	Storage Period	FL1879	FL2053	Snowden
Bud end	C	Before	0.94±0.34a	1.73±0.25a	1.56±0.38a
	C	After	0.93±0.23a	1.56±0.25b	0.92±0.16b
	V	Before	1.18±0.29b	1.66±0.30a	1.87±0.54c
	V	After	0.84±0.26a	1.61±0.22b	1.11±0.28d
Stem end	C	Before	1.19±0.36b	1.64±0.34a	1.36±0.56e
	C	After	1.30±0.32b	1.49±0.20c	0.95±0.21b
	V	Before	1.62±0.69c	1.66±0.32a	1.72±0.75c
	V	After	1.12±0.31b	1.48±0.24c	1.10±0.23d

Table 3.4b: Bud-end and stem-end sucrose content (mg g^{-1} FW) of tubers from fumigated field (C) or non-fumigated field (V) before and after post harvest storage at 13°C for two months in 2011. Within each column, values followed by the same letter did not differ significantly using LSD at p=0.05.

Location	Treatment	Storage Period	FL1879	FL2053	Snowden
Bud end	C	Before	1.07±0.42a	1.30±0.24a	1.18±0.34a
	C	After	0.85±0.18b	1.27±0.20a	0.90±0.16b
	V	Before	2.05±1.86c	1.54±0.59b	1.09±0.27a
	V	After	1.10±0.25a	1.10±0.14c	0.98±0.37b
Stem end	C	Before	1.28±0.70d	1.45±0.53b	1.30±0.28c
	C	After	1.42±0.40d	1.42±0.42b	1.27±0.27c
	V	Before	1.99±1.46e	2.00±1.17d	1.40±0.39d
	V	After	1.62±0.48f	1.45±0.31b	1.36±0.43c

Table 3.5a: Bud-end and stem-end glucose content (mg g^{-1} FW) of tubers from fumigated field (C) or non-fumigated field (V) before and after post harvest storage at 13°C for two months in 2010. Within each column, values followed by the same letter did not differ significantly using LSD at p=0.05.

Location	Treatment	Storage Period	FL1879	FL2053	Snowden
Bud end	C	Before	0.04±0.05a	0.03±0.01a	0.04±0.02a
	C	After	0.07±0.06a	0.24±0.44b	0.12±0.13b
	V	Before	0.31±0.47b	0.04±0.02a	0.03±0.01a
	V	After	0.35±0.73b	0.06±0.09a	0.16±0.22b
Stem end	C	Before	0.12±0.40a	0.08±0.09a	0.13±0.21b
	C	After	0.52±0.77c	0.82±0.68c	0.56±0.35c
	V	Before	0.28±1.27b	0.07±0.08a	0.04±0.03a
	V	After	0.53±0.75c	0.44±0.35d	0.48±0.40c

Table 3.5b: Bud-end and stem-end glucose content (mg g^{-1} FW) of tubers from fumigated field (C) or non-fumigated field (V) before and after post harvest storage at 13°C for two months in 2011. Within each column, values followed by the same letter did not differ significantly using LSD at p=0.05.

Location	Treatment	Storage Period	FL1879	FL2053	Snowden
Bud end	C	Before	0.02±0.01a	0.04±0.01a	0.04±0.02a
	C	After	0.07±0.03a	0.18±0.38b	0.35±0.13b
	V	Before	0.06±0.10a	0.04±0.02a	0.04±0.02a
	V	After	0.29±0.93b	0.08±0.17a	0.11±0.08c
Stem end	C	Before	0.05±0.14a	0.07±0.09a	0.11±0.21c
	C	After	0.21±0.15b	0.88±0.70c	0.71±0.38d
	V	Before	0.08±0.12a	0.08±0.16a	0.09±0.15a
	V	After	0.13±0.09c	0.23±0.28b	0.47±0.33e

Under both fumigation treatments at harvest and out of storage, defect score had a relatively strong correlation with stem-end glucose content in all the three varieties (Table 3.6). There were no strong correlations between severity of defect and stem-end sucrose contents, as well as the ratio of stem-end glucose to sucrose. Tuber sucrose,

glucose or the ratio of glucose to sucrose on the bud end of tubers did not correlate with defect score (data not shown).

Table 3.6: Correlation coefficients of correlation analysis of relationship between defect score and sucrose, defect score and glucose, as well as defect score and the ratio of glucose to sucrose on the stem end of tubers in each variety.

Variety	Location	Score~Suc	Score~Gluc	Score~Glu/Suc
FL1879	Bud end	0.10	0.09	0.01
	Stem end	0.11	0.37	0.03
FL2053	Bud end	0.16	0.08	0.04
	Stem end	0.05	0.45	0.15
Snowden	Bud end	0.11	0.16	0.08
	Stem end	0.07	0.39	0.01

Higher stem-end chip defect scores correlated with higher acid invertase activity

Acid invertase activities on the stem end of FL1879 tubers from non-fumigated fields were higher in tubers that produced chips with defect score 4 and 5than in tubers with defect scores of 0-3 (Fig. 3.2). For tubers from fumigated fields, higher acid invertase activities were noted on the stem end of tubers that had defect scores of 3, 4 and 5relative to tubers with scores of 0-2 (Fig. 3.3). No obvious differences were noted between invertase activities quantified with or without the enzyme inhibitor (Fig. 3.2 and 3.3). There was a difference between average stem-end invertase activity from fields with higher and lower *V. dahliae* pressure before and after storage (Fig. 3.4, $p<0.05$). Invertase activities were higher in tubers out of storage than those at harvest under both treatments (Fig. 3.4, $p<0.05$). The increased activities were observed most in tubers with defect scores 3, 4 and 5 (Fig. 3.2 and 3.3). Invertase activities in tubers that

produced chips with defect score 0 were significantly lower than those with other defect scores in both treatments (Fig. 3.2 and 3.3, $p<0.05$). Bud end acid invertase activities did not significantly correlate with defect scores (data not shown).

Fig. 3.2: Invertase activities assayed with inhibitor *(black and dark grey bars)* and without inhibitor (*white bars, light grey bars*) at harvest (*black and white bars*) and out of storage (*dark grey and light grey bars*) on the stem end of tubers of FL1879 from the fumigated field. Error bars are standard deviations of tubers that produce chips with the respective defect score.

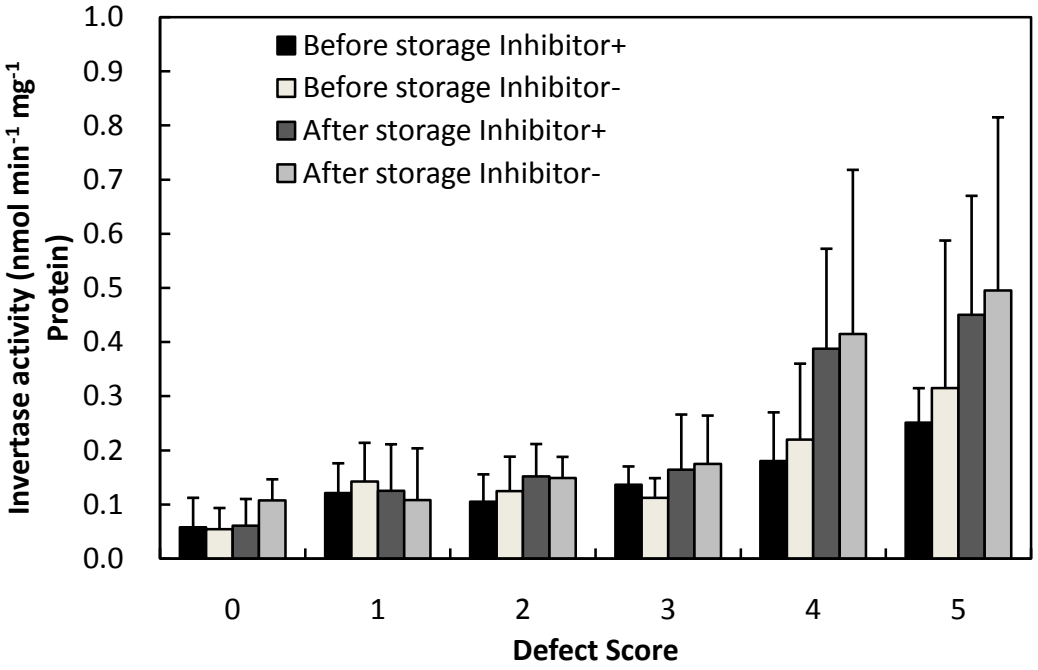


Fig. 3.3: Invertase activities assayed with inhibitor (*black and dark grey bars*) and without inhibitor (*white bars, light grey bars*) at harvest (*black and white bars*) and out of storage (*dark grey and light grey bars*) on the stem end of tubers of FL1879 from the non-fumigated field. Error bars are standard deviations of tubers that produce chips with the respective defect score.

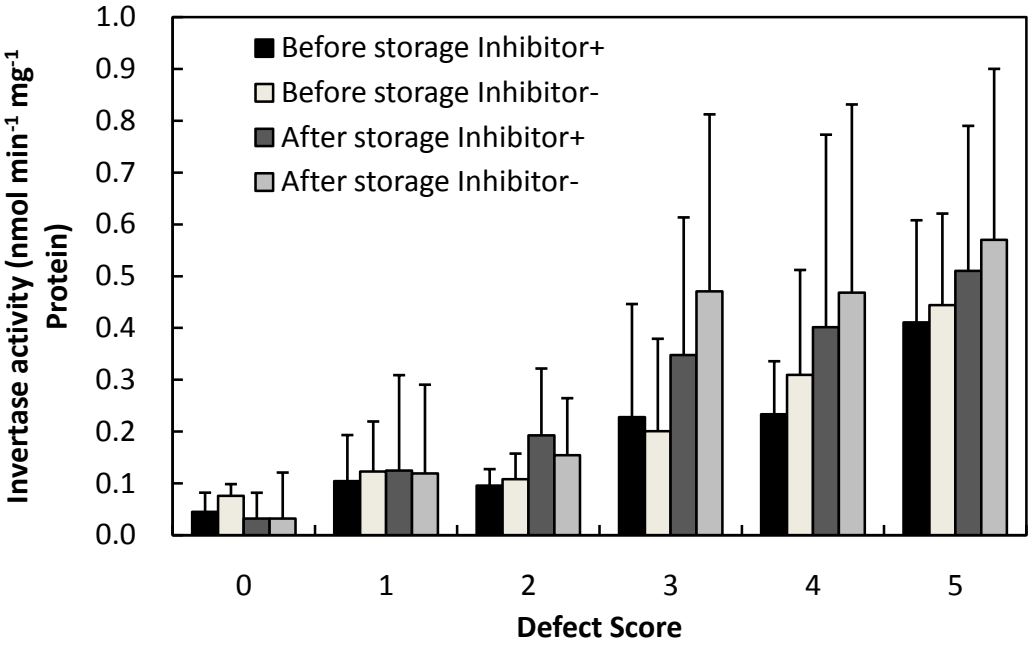
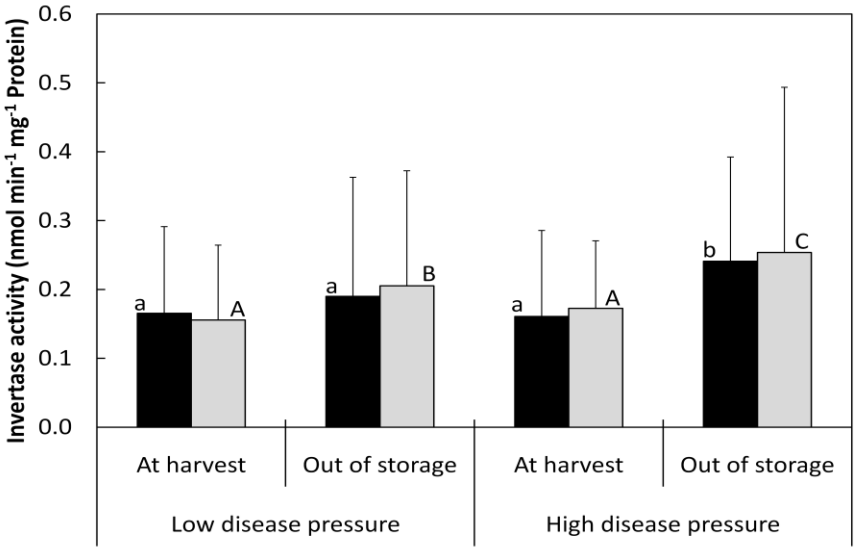


Fig. 3.4: Average stem-end invertase activities measured without inhibitor (*black bars*) and with inhibitor (*grey bars*) at harvest and out of storage in the tubers of FL1879 grown in non-fumigated and fumigated fields. Error bars are standard deviations of thirty samples. Bars with the same letter did not differ significantly using LSD at p=0.05.



Discussion

Severe stem-end chip defects were observed in both fumigated and non-fumigated fields in all the three varieties, although incidence in the fumigated field was lower than that in the non-fumigated field (Table 3.2). This observation raises two points related to the causes of stem-end chip defect. First, environmental stress (Wang *et al.* 2012) and the pathogen stress from *V. dahliae* may both have contributed to the development of stem-end chip defect. The growing seasons in 2010 and 2011 were both characterized by periods of hot weather that produced stem-end defect chips in many varieties in Central Wisconsin. Previous data showed that heat stress might increase stem-end chip defect severity. Warm temperatures favor growth of the fungus in the xylem while at the

same time increasing the need for transpiration by the plant. It is possible that the fungus exacerbates the influence of high temperature stress on tuber carbohydrate metabolism, increasing the severity of the defect in chips that fry dark (Table 3.2). Secondly, other fungal or bacterial pathogens, in the soil perhaps with the aid of nematodes, may be contributing to stem-end chip defect. Soil is a complicated and integrative system. Many organisms that can exert some effects on the vascular dysfunction of potato tubers during the growing season or out of storage may be possible candidates. Fumigation is relatively indiscriminate with regard to the organisms that it kills. Hence, organisms that co-infect plants along with *V. dalhiae* remain candidates for making a contribution to stem-end chip defect. More research needs to be performed to address this issue in depth.

Verticillium CFU data across the two years demonstrate that for the plants in the non-fumigated field, CFU values from early season fresh stem sap to final harvest dry stems were always higher than for plants in the fumigated field (Fig. 3.1). Johnson and Dung (2010) stated that although infection may occur early in the growing season, Vw symptoms generally do not develop until the later part of the growing season when rapid tuber bulking occurs and the fungus has been spread and colonized the whole plant. This is consistent with the observed peak CFU values in all the varieties at the late plant growth stage (Fig. 3.1).

Higher stem-end chip defect scores were correlated with higher acid invertase activities at the tuber stem end (Fig. 3.2 and 3.3). This finding confirms and extends the observation described in Chapter 2 that high rates of acid invertase were associated with

more severe stem-end chip defects resulting from high environmental stress. Low defect scores of 0, 1, and 2 had no apparent relationship with stem-end acid invertase activities in the previous study, but in the present study, tubers with defect score 0 had significantly lower acid invertase activity than tuber which defect scores of 1 or 2. For defect scores of 3, 4 and 5, average invertase activities on the stem end of tubers that were harvested from the non-fumigated field were higher than those from the fumigated field (Figs. 3.2 and 3.3). Furthermore, the increase in invertase activity that occurred during storage (Fig. 3.4) was greater for tubers harvested from the fields with higher disease pressure. Taken together, these data suggest that *V. dahliae* infection of plants could enhance the up-regulation of invertase activity and result in higher accumulation of reducing sugars later in the storage period.

Sucrose is the major sugar used by potatoes for transport and provides the energy needed for tuber growth and starch synthesis (ap Rees and Morrell 1990). The elevated sucrose contents on both ends of tubers more highly infected by *V. dahliae* (Table 3.4) are likely a result of the plant`s response to infection. *V. dahliae* in xylem vessels could reduce transpiration and thus produce a stress signal for starch degradation in the basal end of underground tubers as has been observed for Russet Burbank (Krauss and Marschner 1984). Source-sink relationships between different organs of the plant dictate carbohydrate partitioning and organ growth rate (ap Rees and Morrell 1990; Dwelle 1985). Pathogen stress can disturb these source-sink relationships and disrupt normal potato growth as well as starch deposition (Basu and Minhas 1991; Krauss and Marschner 1984; Sowokinos 1990; Wolf *et al.* 1990, 1991). Alternatively, early wilting of

plants in non-fumigated fields caused great decrease of canopy cover, and thus plants were exposed to higher risk of heat stress during the day, which can possibly result in increase of sucrose contents in tubers. In addition, early dying of plants might cause tuber chemical immaturity at harvest, where sucrose content is higher than an accepted level for good fry qualities (usually 1.0 mg g^{-1} FW, Bussan *et al.* 2009b).

Why for FL2053 and Snowden the bud-end sucrose at harvest and out of storage was lower than the stem-end sucrose in one year but higher in the other year (Table 3.4a, b) is not known. One possible reason might be that there are enzymes in addition to acid invertase that are involved in changing the sucrose content in tubers in response to stresses. Those enzymes could include starch phosphorylase, UGPase, and sucrose synthase in the carbohydrate metabolism pathway shown in Fig. 1.2.

Stem-end chip defect score had a relatively strong correlation with stem-end but not bud end glucose contents in all varieties (Table 3.6). This was consistent with the observed differences in invertase activity between the tuber stem and bud ends.

In summary, it is concluded that colonization of the xylem by *V. dahliae* during the growing season contributed to an up-regulation of acid invertase activity and increase in sucrose on the stem end of tubers which subsequently lead to an accumulation of reducing sugars that were the primary cause of dark pigment formation in defect chips.

Both Vw and stem-end chip defect are serious concerns for the U.S. potato chipping industry. Selecting chipping varieties resistant to Vw and efficiently monitoring environmental conditions, especially temperature changes, during crop growth are two possible effective approaches to reduce impacts of Vw disease as well as stem-end chip

defect, and thus to reduce the financial risks to growers and processors.

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Chapter 4: Conclusions and future work

Two types of factors were identified to cause stem-end chip defect: abiotic and biotic factors. For the first type, the influences of moderate heat stress in combination with or without soil moisture deficit, stresses during early or late tuber bulking stages, tuber immaturity caused by delay of vine senescence, as well as post harvest storage of tubers at 13°C for two months were investigated for two years in the UW Biotron. Among all these factors, only moderate daytime heat stress at 30°C increased the incidence of severe stem-end chip defects in one of two years. From this we concluded that moderate abiotic factors are not consistently sufficient to lead to stem-end defect formation and development. A follow up study showed that more severe daytime heat stress at 35°C combined with or without nighttime heat stress at 30°C was able to induce high rates of stem-end defect occurrence. It is likely that the increase in heat stress severity caused the defect.

For the second type, our two-year field research has indicated that the vascular fungus *V. dahliae* may contribute to stem-end chip defect development. Higher incidence of defect, higher sucrose and glucose contents, and higher invertase activities were observed in plants that were more severely infected by *V. dahliae* during the growing season.

There may be interactions between the abiotic and biotic factors. Plants under low Vw disease pressure that suffered from a short period (about ten days) of heat stress with daytime temperature over 30°C in the summers of 2010 and 2011 developed chips with stem-end defect. Other plants exposed to high Vw pressure developed a higher

percentage of tubers that produced stem-end defect chips. In production fields, it may be impossible to completely isolate the abiotic factors from the biotic ones.

Our conclusion so far is that stem-end chip defect may be mainly caused by high temperature stress in combination with or without vascular pathogen *V. dahliae* during the growing season. There remain some questions that need to be addressed further:

1: More research is needed to deeply explore the underlying biochemical nature of stem-end chip defect. We have shown that elevated invertase activities and glucose contents were observed in tissues with high stem-end chip defect scores both at final harvest and out of storage. Glucose, produced from the invertase-catalyzed breakdown of sucrose, is the major component participating in the Maillard reaction that results in dark pigments on the chips. It is possible that severe heat stress during the growing season is a signal that might up-regulate invertase activities, and possibly alter other enzyme activities in the carbohydrate metabolic pathway, and switch tubers from starch synthesis to starch degradation and reducing sugar accumulation. Stem-end chip defect was not noted immediately after heat stress or at harvest, so there may be a delayed response of potato tubers to the environmental stress in terms of developing this disorder.

Research could include measuring changes in activities of other enzyme that are involved in the tuber carbohydrate metabolism pathways (sucrose synthase, starch phosphorylase, UDP-Glucosepyrophosphorylase, etc), compositional changes (total tuber solids, tuber starch, inorganic Pi, and pH) in stem-end defect tissues, as well as the relative physiological properties of tubers that produce stem-end chips (respiration rate,

cell membrane permeability, tissue growth rate).

2: Some work is necessary to investigate the underlying mechanism of how *V. dahliae* contributes to stem-end chip defect. Higher incidence of defect, higher sucrose and glucose contents, higher invertase activities were observed in plants that were more severely infested by *V. dahliae* during the growing season (Chapter 3). How the colonization and translocation of *V. dahliae* within the plant vascular system is connected with those changes in plants is a question that needs to be answered.

3: More research should be conducted on the long-term storage of tubers with stem-end chip defect. Recent blanching experiments not described here demonstrated that tubers that were or were not infested by *V. dahliae* during the growing season developed stem-end discoloration that took longer than four hours to be removed in 60°C ddH_2O. After three months of storage at 13°C, it took about two to three hours to remove the dark color, and after more than four months of storage, it only took about one hour to completely remove the reducing sugars in the slices before frying for chips. The relatively shorter time to blanch the precursors of dark color away from the slices may dictate that longer-term storage at a warm temperature can help to improve stem-end defect on chipping potato tubers.

4: More work should be done about the effective management practices of stem-end chip defect. High temperatures in summer are unavoidable in commercial production of raw materials for chip industry, and thus regularly monitoring of the soil temperature changes is helpful to predict probability of stem-end defect for chipping potato growers.

Appendix 1: One year data for FL1922 and FL0001 in the *Vert* field trials

FL1922 was planted in 2010 and FL0001 was planted in 2011 for studying the effects of *Verticillium dahliae* infection on stem-end chip defect. The one year data for the two varieties is presented as below.

Table A1.1: Average length of primary stems per plants in FL1922 and FL0001 at the early season harvest, number of tubers produced in each plant at the late season harvest and final harvest, tuber yields in each plant at final harvest of each variety in fumigated field (C) or non-fumigated field (V). Values followed by different letters significantly differ from each other by Student`s t-test at p=0.05.

Variety	Treatment	Average length of primary stems per plants in season (cm)	Number of tubers produced per plant in season	Number of tubers produced per plant at harvest	Tuber yield per plant at harvest (Kg)
FL1922	**C**	67±11a	13±5a	11±5a	1.6±0.5a
	V	50±9b	8±4b	8±4a	1.2±0.5b
FL0001	**C**	81±6a	16±7a	15±6a	2.4±1.1a
	V	62±7b	11±4b	13±5a	2.1±0.5a

Table A1.2: Percentage of severe stem-end chip defect in FL1922 and FL0001 in fumigated field (C) or non-fumigated field (V) at four different sampling time points. At each time point, values followed by the same letter in each treatment differ significantly using Chi-square test at p=0.05.

Variety	Treatment	Early Season Harvest	Late Season Harvest	Final harvest	Out of storage Sampling
FL1922	**C**	0a	2a	27a	45a
	V	0a	27b	14a	13b
FL0001	**C**	0a	0a	50a	57a
	V	0a	13b	57a	73b

Fig. A1.1: Mean log10-tranformed Colony Count Unit (CFU) in fumigated field (*black bars*) or non-fumigated field (*grey bars*) at early season, late season and final harvest for FL1922 (a) and FL0001 (b). Error bars are standard deviations of 20 plants in each variety under each treatment.

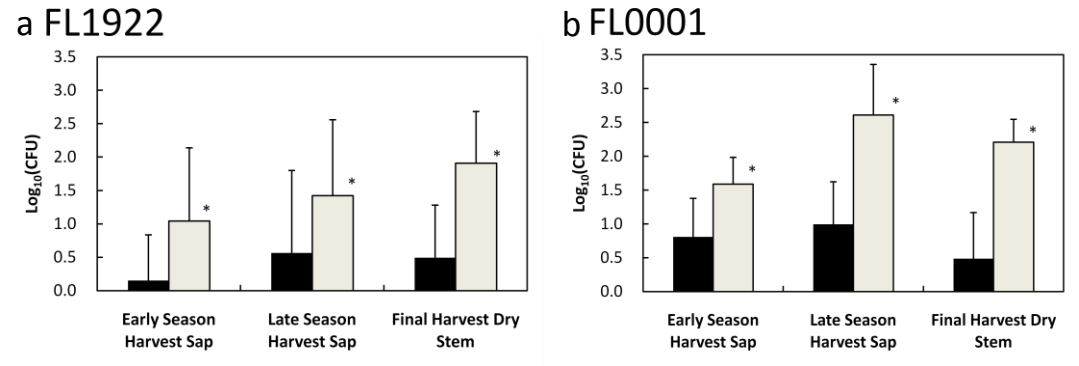

Table A1.3: Bud-end and stem-end sucrose content (mg g^{-1} FW) of tubers from fumigated field (C) or non-fumigated field (V) before and after post harvest storage at 13°C for two months for FL1922 and FL0001. Within each column, values followed by the same letter did not differ significantly using LSD at p=0.05.

Location	Treatment	Storage Period	FL1922	FL0001
Bud end	C	Before	1.47±0.33a	2.12±1.26a
	C	After	1.15±0.38b	0.95±0.16b
	V	Before	1.94±0.57c	1.56±0.54c
	V	After	1.26±0.28b	1.00±0.17b
Stem end	C	Before	1.72±0.76d	1.81±0.55d
	C	After	1.98±0.85c	0.85±0.14b
	V	Before	2.21±0.56e	1.48±0.40c
	V	After	1.56±0.46a	0.90±0.14b

Table A1.4: Bud-end and stem-end glucose content (mg g^{-1} FW) of tubers from fumigated field (C) or non-fumigated field (V) before and after post harvest storage at 13°C for two months for FL1922 and FL0001. Within each column, values followed by the same letter did not differ significantly using LSD at p=0.05.

Location	Treatment	Storage Period	FL1922	FL0001
Bud end	C	Before	0.03±0.01a	0.11±0.23a
	C	After	0.05±0.06a	0.12±0.07a
	V	Before	0.04±0.01a	0.04±0.01b
	V	After	0.05±0.04a	0.06±0.02b
Stem end	C	Before	0.05±0.11a	0.73±1.01c
	C	After	1.07±1.49b	0.89±1.10c
	V	Before	0.05±0.05a	0.10±0.13a
	V	After	0.24±0.38c	0.33±0.43d

Appendix 2: *Verticillium dahliae* DNA in the stem-end chip defect tissues

Infection of *Verticillium dahliae* in the vascular tissues of the potato plants can significantly induce greater incident of stem-end chip defect. Since the conidia of *V.* specifically reside within the vasculature of the host plants, it can be hypothesized that there is a relationship between the amount of *V. dahliae* DNA present in the vascular tissues of tubers and the severity of stem-end chip defects on the chips.

Introduction

As described in Chapter 3, *Verticillium dahliae* appears to play a role in the formation and development of stem-end chip defect. Higher pressures of Vw disease can cause higher incidence of defect by up-regulating acid invertase activity on the stem end of tubers. However, the relationship between level of the fungal pathogen infection within the vasculature of potato tissues and the severity of stem-end chip defect is still unknown. *V. dahliae* might promote defect formation as a direct consequence of its presence in the tuber vasculature. Alternatively, *V. dahlia* may exert its effect through production of a toxin that is mobile in the xylem. One way to determine if the pathogen is present in the vasculature of tubers is to see if pathogen DNA is present in the affected tissues. The objective of this research was to extract *V. dahliae* DNA from the stem-end chip defect tissues and correlate the presence of *V. dahliae* DNA with the severity of defect on the chips.

Materials and methods

Plant materials. Eight tubers in FL1879, FL2053 and Snowden, four from the fumigated field and four from the non-fumigated field as described in Chapter 3, were cut into halves through the stolon attachment point, and a slice that was 1 mm in thickness was cut off one half and discarded. A second slice was cut, fried and scored for stem-end chip defect severity. Stem-end vascular tissues from the two halves were collected with a surface-disinfested razor blade, placed into a paper bag for lyophilizing for four days.

DNA extraction. DNA was extracted from the freeze-dried tissues using published protocol by Wulff *et al.* (2002). It was expected that most of the extracted DNA would originate from *Solanum tuberosum*, but DNA of *V. dahliae* would be extracted at the same time if the pathogen is present in the sampled tissues. DNA concentrations in the extracts were 500 to 2000 $ng \bullet ul^{-1}$, and these were diluted to approximately 50 $ng \bullet ul^{-1}$ prior to PCR amplification.

Primer design and PCR amplification. As a marker for DNA of *S. tuberosum*, a primer pair that amplifies a fragment of 115 bp in the actin (*act*) gene protein-coding region was used (Atallah *et al.* 2007). For *V. dahliae*, a primer pair that amplifies a fragment of 261 bp in the β-tubulin 2 protein-coding sequence was used (Table A2.1). PCR amplifications with both primer pairs were conducted in a Bio-Rad iCycler thermocycler (Hercules, CA). The 20-µl amplification reactions for amplifying *S. tuberosum* DNA included 100 ng of total extracted DNA and 200 nM each primer in Takara General reaction mixture for PCR (Takara Bio INC.) (1.5 mM $MgCl_2$, 2.5 mM each dNTPS, and *Taq*

polymerase at 5 U•µl^{-1}). The 20-µl amplification reactions for amplifying *V. dahliae* DNA included 200 ng of total extracted DNA and 200 nM each primer in Takara General reaction mixture for PCR (1.5 mM $MgCl_2$, 2.5 mM each dNTPS, and *Taq* polymerase at 5 U•µl^{-1}). The following amplification protocol was used for both reaction systems: initial denaturation for 3 min at 95°C, then 40 cycles of 95°C for 10 s, 58.5°C for 35 s, and renaturation for 20 s at 72°C. Amplified products were visualized in 1.5% agarose gels.

Target organism	Gene target	Primer	Sequence
Verticillium dahliae	*β-tubulin*	VertBt-F	CTGACCTTGGTTTCCCTCTCTCTT
		VertBt-R	TGTAGCTGTCATCGCGTCAGTAAC
Solanum tuberosum	*actin*	PotAct-F	TGAACACGGAATTGTCAGCA
		PotAct-R	GGGGTTAAGAGGGGCTTCAG

Table A2.1: Primers developed to defect *Verticillium dahliae* and *Solanum tuberosum* (potato) DNA.

Results and discussion

Primers designed to amplify potato DNA produced the expected 115 bp product from all the 16 samples (Fig. A2.1). There was no apparent relationship between band width and brightness of the *S. tuberosum* DNA products from different tissues with different defect scores as indicated in Table A2.2.

A 261 bp fragment amplified from *V. dahliae* DNA was not uniformly present in all 16 samples (Fig. A2.2). Pathogen DNA was not amplified from bud end and stem end

vascular tissues in tubers from the fumigated field when tubers produced chips with defect score of 0, 1, and 2. In contrast, both bud end and stem end vascular tissues contained the pathogen DNA in tubers from the non-fumigated field when tubers produced chips with defect score of 3 and 5.

Fig A2.1: PCR products of DNA from *S. tuberosum* (potato). Lane 17 and 18 are negative controls with pure water as the template. Lane 19 and 20 are positive controls with pure potato DNA (50 ng•µl^{-1}) as the template.

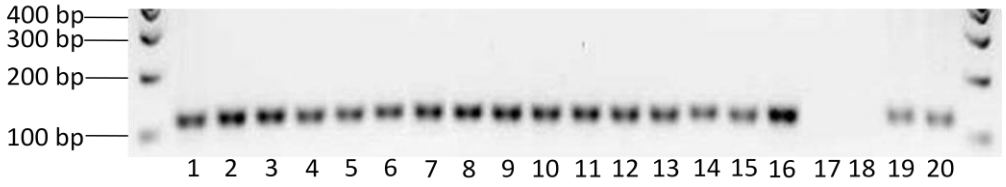

Fig A2.2: PCR products of DNA from *V. dahliae*. Lane 17 and 18 are negative controls with pure water as the template. Lane 19 and 20 are positive controls with DNA from a pure culture of *V. dahliae* DNA (50 ng•µl^{-1}) as the template.

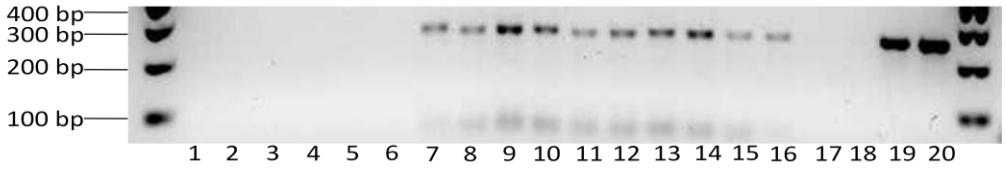

Table A2.2: Bud-end (B) and stem-end (S) tissues from tubers of FL1879, FL2053 and Snowden in the fumigated (C) and non-fumigated (V) fields were extracted for DNA. Stem-end chip defect score is listed on the right-most column.

Lane #	Variety	Treatment	Sampling Location	Defect score
1	**FL2053**	C	B	0
2	**FL2053**	C	S	0
3	**Snowden**	C	B	1
4	**Snowden**	C	S	1
5	**FL1879**	C	B	2
6	**FL1879**	C	S	2
7	**Snowden**	C	B	3
8	**Snowden**	C	S	3
9	**FL2053**	V	B	3
10	**FL2053**	V	S	3
11	**FL1879**	V	B	3
12	**FL1879**	V	S	3
13	**FL2053**	V	B	5
14	**FL2053**	V	S	5
15	**Snowden**	V	B	3
16	**Snowden**	V	S	3

These data support the hypothesis that *V. dahliae* is present in the vasculature of potato tubers, but questions remain to be answered. For example, vascular tissues from tubers harvested in the fumigated field that produced stem-end chip defect scores of 3 (lanes 9-13) contained *V. dahliae* on both bud and stem ends. Whether this was due to pathogen infection of tubers tissues during the growing season or continued growth or movement of the fungus after harvest is unknown. Likewise, it is not known if there is a differential response of stem-end and bud-end tuber tissues to infection that leads to a defect at the stem and but not the bud end. Care was taken to avoid cross contamination of samples during the DNA extraction, but this possibility needs to be further investigated. Atallah *et al.* (2007) indicated the lowest concentration of *V. dahliae* DNA reliably amplified was 148 fg (1.48×10^{-4} ng), so PCR is able to detect a low amount of contaminated *V. dahliae* DNA in the samples. It is recommended that techniques be developed to specifically and precisely cut vascular tissues away from the whole tuber

for DNA extraction. Fig. A2.2 hints that there might be a correlation between the amount of pathogen DNA present in the vascular tissues and the intensity of bands on the gel. This correlation is dependent in part on the relative ratio of sampled vascular tissues to other tissues in the sample.

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Appendix 3: Similarities and differences between stem-end chip defect and sugar-end defect

In North America, processing quality is of high importance to the potato industry. Approximately 47% of the potato crop is utilized for making chips and frozen-fried products (National Potato Council 2006). Consumer preference continues to switch from baked or home cooked potatoes to processed products over the last decade (The Deliberate Agrarian 2006). Stem-end chip defect is one of the most serious quality concerns for the potato chip industry in the US. French fries are the other main processed potato product and they are served in restaurants worldwide. The world's appetite for factory-made French fries has been put at more than 11 million tons a year (International Year of the Potato 2008). Sugar-end defect is a critical quality concern for French fries production. The losses caused by this one defect have not been quantified, but can be serious due to crop rejection and increase of processing cost (Thompson *et al.* 2008).

These two major quality problems of processed potato products, stem-end chip defect and sugar-end defect, have many similarities and differences. These similarities and differences are described in this chapter in an attempt to discern if they are fundamentally the same defect, with differences in appearance depending on the tuber market class.

Similarities between stem-end chip defect and sugar-end defect

Symptoms are similar

Both defects are typified by dark fry color on the stem (basal) end of potato products. Dark colors extend a certain distance (1.3 cm or more for both defects) from the stolon attachment point into the internal tissues, while the reminder of the product would be acceptably light based on standardized color evaluation indexes (The index developed by Bussan *et al.* in 2009 for stem-end chip defect and the USDA color standard for frozen French fries).

High levels of reducing sugars on the stem end are the direct cause

Both defects are caused by high levels of reducing sugars on the stem end of tubers. These react with free amino acids and other cellular substrates in the Maillard reaction during frying (Gould and Plimpton 1985; Shallenberger *et al.* 1959). Examples of compounds produced in Maillard reaction include Strecker aldehydes and alkylpyrazines, which are the major flavor components of fried potatoes (Morales *et al.* 2008), and melanoidins that contribute dark brown color (Damodaran *et al.* 2008).

Elevated invertase activity on the stem end of tubers leads to higher accumulation of reducing sugars

Invertase is an enzyme that breaks down sucrose into the reducing sugars glucose and fructose. The particular role that reducing sugars play in the dark color formation of the

two defects makes it important to understand the biochemical changes of invertase activity under scenarios for developing both defects. Previous data have shown that higher severity of stem-end chip defect correlated with higher acid invertase activities (Fig. 2.5). Sowokinos *et al.* (2000) demonstrated that sugar-end tissues displayed a 4.2-fold increase in total acid invertase activity (inhibitor destroyed). The net result of the changes in enzyme activity in the defect tissues appeared to shift carbohydrate metabolism away from starch synthesis towards starch mobilization and accumulation of reducing sugars (Thompson *et al.* 2008).

Cold temperature is not required for the development of the two defects

One other major quality concern for the processed potato industry is called cold-induced sweetening, where tubers stored at less than about 10°C have accelerated rates of conversion of starch to reducing sugars (Sowokinos 2001), which results in dark-pigmented potato products after frying in oil at high temperature. However, since stem-end chip defect or sugar-end defect can be observed in tubers that are stored at 13°C, it is clear that these two defects are not the consequences of cold-induced sweetening. Cold-induced sweetening is typically a problem that happens during post harvest storage of potato tubers, whereas stem-end chip defect and sugar-end defect are problems caused by certain factors prior to harvest.

Differences of stem-end chip defect and sugar-end defect

Different histories

Stem-end chip defect has only recently become a large concern for chip manufacturers in U.S. To our knowledge, peer-reviewed manuscripts on stem-end chip defect have not been published. Sugar-end defect was recognized in the literature as a problem for more than 70 years (Lugt 1960; Nielsen and Sparks 1953; Penman 1929; Sparks 1958), and has been the subject of considerable research for decades. More than a hundred peer-reviewed reports have been published about sugar-end defect worldwide.

Different potato varieties are associated with each defect

Stem-end chip defect occurs on many chipping potato varieties including Snowden and the Frito-lay varieties used in this research. On the contrary, sugar-end defect happens on varieties that are used for French fry production, most notably Russet Burbank.

Different appearance

Stem-end chip defect is associated with a localized discoloration focusing on the vasculature and penetrating to the adjacent tissues on the stem-end portion of chips. Sugar-end defect is characterized by a dark blemish area that includes the vascular tissues at the strip`s stem end, but localization in a specific spot or tissue may not occur.

Biochemical characteristics are different

Little differences are noted in the amount of starch at the bud end and stem end of tubers with stem-end chip defect (data not shown). However, sugar-end defect is marked by low starch and high reducing sugars in the stem-end tissue while the younger bud-end tissue has high starch and low reducing sugar content (Eldredge *et al.* 1996; Iritani 1981, 1987; Iritani and Weller 1973, 1980; Kleinkopf *et al.* 1988; Shock *et al.* 1987, 1992, 1993).

Environmental causes of the two defects are different

The exact causes of stem-end chip defect have not been specified, but previous data reported that two-week long periods of warm days (30˚C) increased the incidence of stem-end chip defect in one of two years (Wang *et al.* 2012). Two-week periods of hot days (35˚C) and warm nights (30˚C) increased defect incidence in two of two years (Bethke, personal communication). Hot days and cool nights (18˚C) also increased the incidence of stem-end chip defect in one of two years, although soil temperatures may have remained above nighttime air temperatures. No specific stage during the growing season has been identified yet for maximal defect occurrence. Water deficit at neither plant growth stage is correlated with significant stem-end chip defect incidence. These data indicated that high daytime or nighttime temperatures or both may be the major contributors to stem-end chip defect formation.

By way of comparison, previous literature (Eldredge *et al.* 1996; Shock *et al.* 1987, 1989,

1992, 1993, 1998; Stieber and Shock 1991; Stieber *et al.* 1988, 1989) concluded that drought and heat stress (27-30°C of daytime temperature) at the early tuber bulking stage were the direct cause of sugar-end defect. Because drought and heat stress tend to occur together, it has been difficult to separate their roles in sugar-end development. A single transitory water deficit was sufficient to cause sugar-end defect and was more damaging than season-long stress (Thompson *et al.* 2008).

Associations of soil fertility with defect development are different

Soil fertility has a major impact on the rate of vine senescence. Although for both defects, samples collected from plants with senesced vines had lower sucrose contents compared to those from plants with green vines, Wang *et al.* previously showed that stem-end chip defect incidence is not related with soil fertility and vine senescence. However, early vine death caused by inadequate soil fertility has been shown to increase the incidence of sugar-end defect (Iritani and Weller 1978).

Effects of environmental stresses on sugar changes in tubers with the two defects are different

According to what has presented previously, for chipping potatoes that can potentially develop stem-end chip defect, tuber sucrose content at the end of the late tuber bulking stress period was higher under water stress at a daytime temperature of 30˚C compared to 22˚C, and soil water deficit at early or late tuber bulking state did not significantly correspond with sucrose content change in tubers (Fig. 2.5). In comparison, Bethke *et al.*

(2009) showed that increasing severity of water stress was associated with an increase in tuber sucrose content especially at the early stress periods. Tubers from water-stressed plants at 30°C had higher sucrose contents on average than those of tubers from plants at 22°C. The effect of warmer temperatures was more pronounced at early stress periods and under increasing moisture stress.

Fungal pathogen is involved in stem-end chip defect but not sugar-end defect formation

Our results showed that *V. dahliae*, a vascular fungal pathogen that can cause potato early dying, contributes to stem-end chip defect development. Infection with *V. dahliae* can substantially increase stem-end chip defect incidence in some varieties. However, so far there has not been any report about *V. dahliae* involvement in sugar-end defect development. One possible explanation could be that for stem-end chip defect, *V. dahliae* specifically infests the plant vascular system where certain ongoing metabolic activities could be disrupted and thus to cause the subsequent vascular defect problem. Sugar-end is caused by environmental-stress induced (water and heat stresses in particular) starch degradation as a physiological result of disturbed source-sink relationships of sucrose (Thompson *et al.* 2008), and no specific vascular activity is identified to be involved in this process.

Summary

Stem-end chip defect and sugar-end defect decrease the quality of tubers used for chip

and fry processing in the U.S. There exist many similarities and differences between these two quality issues. A comparison between the two is likely to provide insights into the nature of both defects and to establish a framework for future research. We conclude that stem-end chip defect and sugar-end defect are two different physiological problems for processed potato products.

Stem-end chip defect shares some similarities with sugar-end defect in terms of their similar symptoms characterized by dark pigmented area on the stem end portion of potato processed products. The discoloration is caused by reducing sugars that accumulate at the tuber stem end due to elevated acid invertase activity. Unlike cold-induced sweetening that occurs during post harvest storage under low holding temperatures, those two defects are associated with abiotic and/or biotic stresses during the growing season.

However, stem-end chip defect is different from sugar-end defect in that the related biochemical characteristics and direct causes are not the same. Although glucose content in tissues with either defect is higher than normal tissues, sucrose in sugar-end defect tissues is observed higher than the non-defect tissues. Plants receiving water stress treatment during early bulking had tuber sucrose contents of 4 mg g^{-1} FW (Bethke *et al.* 2009). But there is not any obvious change of sucrose in the stem-end chip defect tissues. Plants receiving water stress treatment during early bulking had tuber sucrose contents of about 2 mg g^{-1} FW (Fig. 2.3). After decades of research conducted about sugar-end defect, its exact causes, a transitory period of water stress with/without heat stress (daytime temperature between 27°C to 30°C) at the early growth stage of potato

plants, are elaborately investigated. Kleinkopf *et al.* (1988) showed that the heat tape treatment increased soil temperature approximately 4°C above ambient and produced 23% sugar-end defects. The combination of water deficit and heat stress increased the incidence of sugar end potatoes to 31%. On the contrary, the research identifying causes of stem-end chip defect is still underway. Recent data has demonstrated that high daytime temperature over 35°C and/or night time temperature over 22°C as well as infection of fungal pathogen *Verticillium dahliae* are considered to play some roles in stem-end chip defect development. Moderate heat stress produced an average 20% stem-end defects (Table 2.3), and the combination of water stress and heat stress produced an average 21% stem-end chip defect (data not shown). Unlike sugar-end defect to which water deficit and soil fertility are contributors, those two stresses are not correlated with stem-end chip defect formation (Table 2.3, no significant increase of stem-end chip defect incidence).

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